RESEARCH ARTICLE

A new polystomatid (Monogenea, Polystomatidae) from the mouth of the North American freshwater turtle *Pseudemys nelsoni*

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Abstract

Based on material collected from *Pseudemys nelsoni* (Reptilia: Chelonia: Emydidae) during a parasite survey of the herpetofauna around Gainesville, Florida, USA, *Polystomoides nelsoni* **sp. n.** is described as a new polystome species. This parasite was found in the oral and pharyngeal region of the host. In a sample of nine *Pseudemys nelsoni*, three specimens were found to release polystome eggs. One turtle was euthanized and dissected and found to be infected in the oral region with 19 specimens belonging to an as-yet-unknown *Polystomoides*. This is only the fifth *Polystomoides* recorded from the Nearctic realm. This species is distinguished from known species by a combination of characteristics including marginal hooklet morphology, body length and haptor dimensions.

Keywords

Introduction

Although monogeneans are predominantly single host fish parasites, polystomatid flatworms (Monogenea, Polystomatidae) radiated onto the tetrapods and are known from a diverse range of hosts, including the Australian lungfish, amphibians, freshwater turtles and the hippopotamus (Raharivololoniaina et al. 2011). Of the 24 currently known polystome genera (Du Preez et al. 2014) three exclusively parasitize turtles, namely *Polystomoides* Ward, 1917, *Polystomoidella* Price, 1939 and *Neopolystoma* Price,

1939 (see Du Preez and Moeng 2004, Du Preez et al. 2007, Du Preez et al. 2008, Morrison and Du Preez 2012, Price 1939, Wright 1879, Yamaguti 1963).

Genera of the subfamily Polystomoidinae, including the three polystome genera known from turtles and the genus *Nanopolystoma,* known from caecilians (Du Preez et al. 2008), all have non-confluent gut caeca lacking diverticula and possess skeletal elements inside the haptoral suckers. Chelonian polystomes are further characterised by non-diverticulated gut caeca of equal length and subsequent, absence of prehaptoral or haptoral anastomoses and a compact medial testis. *Polystomoides* is found in the oral cavity, nasal cavity, cloaca and urinary bladder of the host and has two pairs of hamuli, with the outer pair being larger than the inner pair. *Polystomoidella* parasitizes the urinary bladder of turtles and has a single pair of hamuli. *Neopolystoma* is found in the oral cavity, nasal cavity, ocular cavity, cloaca and urinary bladder and has no hamuli.

At present 54 turtle polystome species are known from 55 host species. Although chelonian polystomes have a broad geographical distribution, only seven *Neopolystoma*, two *Polystomoidella* and four *Polystomoides* species are known from the Nearctic realm. The *Polystomoides* species currently known from this region include *Polystomoides coronatum* (Leydi, 1888) Ozaki, 1935 from *Trachemys dorbigni*; *Polystomoides multifalx* Stunkard, 1924 from *Pseudemys concinna* (LeConte, 1830); *Polystomoides oris* Paul, 1938 and *Polystomoides pauli* Timmers & Lewis, 1979, both from *Chrysemys picta*.

During a survey of freshwater turtles around Gainesville, Florida, USA, *Pseudemys nelsoni* (Reptilia: Chelonia: Emydidae) was found to be infected with an as-yet-unknown *Polystomoides*. This paper provides the formal description of this previously unknown parasite.

Material and methods

During April-June 2004 baited crayfish traps were set to capture terrapins in ponds in and around Gainesville, Florida, USA. Captured turtles were individually placed in 20 L plastic buckets with dechlorinated tap water to a depth of about 50 mm. After a period of 24 hours turtles were removed and the water screened for the presence of polystome eggs. The water from the containers in which turtles were housed was poured through two plankton sieves with respective mesh sizes of 500 μm and 100 μm. The first sieve removed the coarse debris in the water while the second retained finer debris and any polystome eggs that might be present. The contents of both sieves were then washed into separate glass Petri dishes and examined under a dissecting microscope. The Petri dish with contents from the course sieve was scanned for adult parasites that may have dislodged, and the Petri dish with contents from the fine sieve was scanned for polystome eggs.

Recovered eggs were removed and incubated at room temperature in Petri dishes containing clean water. Freshly hatched oncomiracidia were collected and mounted semi-permanently using ammonium picrate as mounting medium to clear the parasites and reveal the marginal hooklets. Turtles that were found not to be infected with polystome eggs were screened a second and third period of 24 hours. A single infected

turtle was euthanized by injecting 0.5 mL of sodium pentobarbitone diluted with water (0.5 mL pentobarbitone and 4.5 mL water) straight into the heart. After 15 minutes the specimen was dissected. The cloaca, urinary bladder and accessory bladders as well as the oral cavities, nasal cavities, pharyngeal cavities, eye surface and cavity under the nictitating membrane were examined for polystomes, with the aid of a stereo microscope. The remainder of the turtles were released where collected.

Polystome whole mounts were prepared as follows: individual mature polystome species collected from the host species were fixed under cover-slip pressure in 10% neutral buffered formalin (NBF). Representative sub-adult specimens were fixed in 70% molecular grade ethanol for future molecular studies.

Parasites earmarked for permanent mounts were hydrated using 30% EtOH, stained overnight in a weak aceto-carmine staining solution, gradually dehydrated to absolute EtOH, cleared in a 1:1 ratio mix of absolute ethanol-xylene and then pure xylene, and mounted in Canada balsam. Preparations were studied using a Nikon E800 compound microscope fitted with a Nikon DXM1200 digital microscope camera connected to a PC. Measurements were taken using Eclipse network software (Nikon). Marginal hooklet measurements were obtained from the oncomiracidia that hatched from incubated eggs, following the protocol developed by Du Preez and Maritz (2006).

Results

Turtles screened and polystomes retrieved

Nine Florida red-bellied turtles (*Pseudemys nelsoni*) were collected and screened. Specimens were collected from Lake Griffin, Lake Lochloosa, Lake Orange, and ponds at the U.S. Geological Survey (USGS) research facility in Gainesville.

Levels of infection

Of the nine turtles examined three were found to be infected (prevalence 33%). Only one turtle was dissected and found to have 19 polystomes in the oral region. These specimens were identified as belonging to *Polystomoides;* however, they did not conform to any of the 38 known *Polystomoides* species.

Molecular studies

Material collected was also studied at the molecular level. Based on 18S and 28S rDNA sequences, the newly discovered polystome differs from all other known turtle polystomes for which molecular data are available and occupies a distinct position basal to other Nearctic chelonian polystomes (see Figures 2a and 2b in Verneau et al. 2011).

Systematics

Class: Monogenea Carus, 1863
Order: Polystomatidea Lebedev, 1988
Family: Polystomatidae Gamble, 1896

Polystomoides nelsoni **sp. n.**
http://zoobank.org/757AA55C-4C80-4075-9B57-A297833F70DA
Figs 1, 2

Specimens studied. Morphological description based on ten sexually mature worms. Holotype (NMB 380) nine paratypes (NMB 381–389) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa.

Type host. *Pseudemys nelsoni* (Carr, 1938) sexually mature male.

Type locality. United States Geological Survey USGS-BRD facility, 7920 N.W. 71st St., Gainesville, Florida, USA (29°43'31"N, 82°25'04"W).

Etymology. The species is named after the host.

Site. Mouth.

Description. Based on ten egg-producing adults. The average measurement is given, followed by the range given in parentheses. Measurements are given in micrometres (µm). Larval (oncomiracidia) measurements are given for the marginal hooklets.

Adult: General characteristics given of mature, egg-producing parasite (Figure 1). Body elongated and ellipsoid, total length 5.707 (3.052–7.378), greatest width 2.278 (1.276–2.751), width at vagina 2.270 (1.276–2.739), haptor length 1.310 (912–1.616), haptor width 1.931 (1.232–2.182); haptor length to body length ratio 0.23; six haptoral suckers, mean diameter 564 (148–781), haptors internally supported by an elaborate skeletal structure. Two pairs of hamuli: inner pair 69 (48–95) long with a hamulus hook length of 21 (17–26); outer pair 138 (104–173) long with a hamulus hook length of 22 (19–26). Mouth sub-terminal. False oral sucker 788 (398–1 036) wide; pharynx length 539 (345–917), width 658 (391–881). Intestine bifurcates with no diverticula and no anastomoses present; caeca extend to the end of the body proper and do not join posteriorly nor do they extend into the haptor. Testis compact, mid-ventral, medial, and posterior to ovary (Figure 1); 401 (108–687) long and 564 (148–781) wide. Genital atrium median, ventral, posterior to intestinal bifurcation: 586 (302–816) in length with 123 (108–132) spines, 101 (93–106) long. Ovary, dextral, anterior, 38% of body length; ovary length 251 (102–330), and width 86 (27–124). Short tubular uterus anterior to ovary, containing up to eight eggs; length 227 (182–274), and width 144 (118–194). No intra-uterine development, operculated egg. Vitellarium extends throughout most of the body proper posterior to the pharynx except the central area around the gonads (Figure 1).**Oncomiracidia.** Marginal hooklets were observed and measured on slides prepared from incubated oncomiracidia (Figure 2). Marginal hooklet I found to be 28 (25–30) and hooklets II – VIII 27 (25–29).
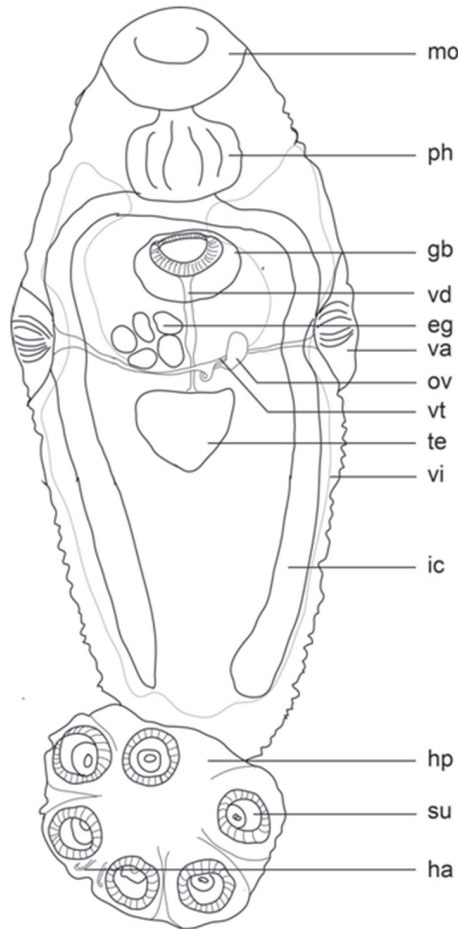
Figure 1. *Polystomoides nelsoni* sp. n. Ventral view of holotype; the dotted line indicates the outline of the vitellarium. Abbreviations: eg, egg; gb, genital bulb; ha, hamulus; hp, haptor; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; va, vagina; vd, vas deferens; vi, vitellarium; vt, vitelline duct. Scale bar: 1 mm.

Remarks. *Polystomoides nelsoni* sp. n. differs from other *Polystomoides* species by a combination of characters. With a body length of 5.707 (3.052–7.378), *P. nelsoni* sp. n. is longer than *Polystomoides megaovum* (2.910), *Polystomoides asiaticus* (4.600), *Polystomoides siebenrockiella* (3.580) and *Polystomoides uruguayensis* (2.560–2.650). However, *P. nelsoni* sp. n. has a shorter body length when compared to *Polystomoides australiensis* (6.193), *Polystomoides fuquesi* (7.480–7.550), *Polystomoides godavarii* (4.200–8.030) and *Polystomoides ludhianae* (6.640–10.060). In terms of the length and width of the haptor, *P. nelsoni* sp. n. (1.310 × 1.931) differs from *P. megaovum* (620 × 880), *P. asiaticus* (1.100 × 1.700), *P. siebenrockiella* (780 × 1.060), *P. australiensis* (1.353 × 2.190) and *P. godavarii* (1.120–1.620 × 1.250–1.710).
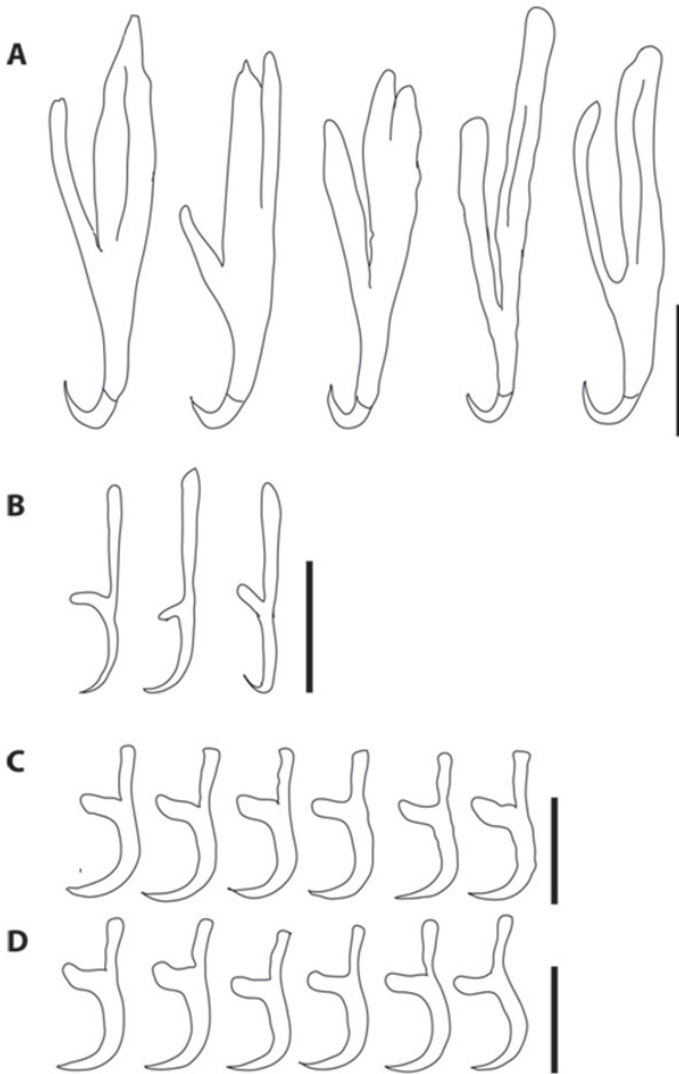
Figure 2. *Polystomoides nelsoni* sp. n. **A** large hamuli from the holotype and paratypes **B** small hamuli from holotype and paratype **C** marginal hooklets 1 **D** marginal hooklets 2–8. Scale bars: 50 μm (**A, B**); 20 μm (**C, D**).

Discussion

All polystome species are host-specific, with chelonian polystomes being strictly site-specific. As a result of this strict site specificity a single host could be infected by more than one polystome species. Chelonian polystomes have been fairly well studied in the USA, with 11 polystomes known from various freshwater turtle hosts (Du Preez and Lim 2000, Morrison and Du Preez 2012).

The two *Polystomoidella* species known from North America are *Polystomoidella oblongum* Wright, 1879 and *Polystomoidella whartoni* Wright, 1879. The seven *Neopolystoma* species known from the USA are: *Neopolystoma elizabethae* Platt, 2000; *Neopolystoma fentoni* Platt, 2000; *Neopolystoma grossi* Morrison & Du Preez, 2012; *Neopolystoma moleri* Morrison & Du Preez, 2012; *Neopolystoma orbiculare* Stunkard, 1916; *Neopolystoma rugosa* MacCallum, 1918; and *Neopolystoma terrapenis* Harwood, 1932. The four *Polystomoides* species known from the USA are: *P. coronatum* Leidy, 1888; *P. multifalx* Stunkard, 1924; *P. oris* Paul, 1938; and *P. pauli* Timmers & Lewis, 1979 (Morrison and Du Preez 2012).

The main feature distinguishing *Polystomoides* from other turtle polystomes is the presence of two unequal pairs of hamuli. The other genera that parasitize turtles either have a single pair of hamuli as in *Polystomoidella* or the hamuli are lacking altogether as in *Neopolystoma*. *Polystomoides* and *Neopolystoma* species can also occasionally be distinguished from *Polystomoidella* in terms of the additional sites (the cavity of the eye and nose, pharynx, cloaca, and mouth) that these species parasitize, as *Polystomoidella* parasites are found to infect only the urinary bladder of their host species.

Polystomoides nelsoni sp. n. can be distinguished from the other *Polystomoides* species by the number of genital spines. *Polystomoides nelsoni* sp. n. has 123 (108–132) genital spines compared to *P. fuquesi* with 2, *P. brasiliensis* with 8–9, *P. bourgati* with 26–29, *P. asiaticus* with 34–40, *P. ludhianae* with 54–64, *P. godavarii* with 64–66, and *P. australiensis* with 74–95. However, *P. multifalx* (120–124) and *Polystomoides stunkardi* (92–109) are two species that also have a large number of genital spines. Compared to *Neopolystoma* species, *Polystomoides nelsoni* sp. n. also has a larger number of genital spines. *Neopolystoma chelodinae* has 14 (12–16), *N. elizabethae* 8 and *Neopolystoma euzeti* 34 (33–36), while *P. oblongum* and *P. whartoni* both have 16 genital spines.

The total length of the genital spines of *Polystomoides nelsoni* sp. n. 101 (93–106) is longer compared to those of other *Polystomoides* species, such as *Polystomoides siebenrockiella* 58 (54–60), *Polystomoides rohdei* 34–52, *Polystomoides platynota*, 60–70, *Polystomoides nabedei* 42–46, *Polystomoides microrchis* 75–88 and *Polystomoides chabaudi* 27 (22–31). The genital spines for *Polystomoides nelsoni* sp. n. are in the same size range as those of *P. australiensis* 93 (78–105). *Polystomoides nelsoni* sp. n. also has larger genital spines compared to those of *Neopolystoma* species, such as *N. chelodinae* 23.6 (20.8–27.2), *N. euzeti* 57 and *N. elizabethae* 10, as well as compared to those of *Polystomoidella* species, such as *P. oblongum* 18–22 and *P. whartoni* 15–18.

Unlike most other polystomes, these parasitizing chelonians have a broad geographical distribution. Both *Neopolystoma* and *Polystomoides* have been reported from the realms around the globe known to be inhabited by freshwater turtles. On the other hand, *Polystomoidella* is mainly known from the Nearctic realm where it is represented by five species. However, Richardson and Brooks (1987) described *Polystomoidella mayesi* from the urinary bladder of a Malaysian box turtle, *Cuora amboinensis*. The presence of *Polystomoidella* in the Oriental realm raises questions of possible misidentifications or a possible parasite transfer. According to Du Preez and Lim (2000) the

possibility of transfer from an introduced American turtle can only be confirmed or refuted if and when *P. mayesi* is found in this chelonian species.

Part of the evolutionary success of chelonian polystomes is the fact that they are site-specific and occupy various sites, including the oral and nasal cavities, eye cavity and the cloaca and urinary bladder. Littlewood et al. (1997) stated that congeneric species infecting the same site in different hosts are more closely related than congeneric species infecting different sites in the same host individuals. The high degree of site specificity allows for speciation and could explain the polystome diversity found in freshwater turtles. With the huge diversity of freshwater turtles globally it is likely that a vast number of chelonian polystomes remain to be discovered.

Acknowledgments

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RESEARCH ARTICLE

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Contributions to the knowledge of oribatid mites (Acari, Oribatida) of Indonesia. 3. The genus *Galumna* (Galumnidae) with description of a new subgenus and seven new species

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Abstract

Seven new species of oribatid mites of the genus *Galumna* are described from litter and soil materials of Sumatra, Indonesia. A new subgenus, *Galumna* (*Atypicogalumna*) **subgen. n.**, is proposed; it differs from all galumnid genera and subgenera by the simultaneous presence of porose areas and sacculi on the notogaster (vs. either porose areas or sacculi present). *Galumna* (*Galumna*) *calva* Starý, 1997 is recorded for the first time in the Oriental region, and *G.* (*G.*) *sabahna* Mahunka, 1995 is recorded for the first time in the Indonesian fauna.

Introduction

This work is a part of a continuing study of the Indonesian fauna of oribatid mites (see Ermilov et al. 2015c, d), and includes data on the genus *Galumna* Heyden, 1826 (Acari, Oribatida, Galumnidae). During taxonomic identification, ten species were found belonging to four subgenera: *G.* (*Atypicogalumna*) subgen. n., *G.* (*Galumna*) Heyden, 1826, *G.* (*Cosmogalumna*) Aoki, 1988 and *G.* (*Neogalumna*) Hammer, 1973. The main goal of the paper is to present data on the specific localities, notes on new records and overall known distribution of registered taxa, and to describe and illustrate a new subgenus and seven new species.

Galumna is a very large genus that was proposed by Heyden (1826) with *Notaspis alatus* Hermann, 1804 as type species. The genus comprises approximately seven subgenera and 180 species (see different opinions: Subías 2004, updated 2015; Ermilov and Anichkin 2014b; Ermilov and Bayartogtokh 2015) having a cosmopolitan distribution (Subías 2004, updated 2015). The subgeneric diagnoses for *G.* (*Galumna*), *G.* (*Cosmogalumna*) and *G.* (*Neogalumna*) were presented by Ermilov et al. (2013), Ermilov and Corpuz-Raros (2015) and Hammer (1973), respectively. The identification keys to selective species of *G.* (*Galumna*) were given by Shaldybina (1975), Balogh and Balogh (2002), Weigmann (2006), Bayartogtokh and Akrami (2014), Ermilov and Anichkin (2014c) and Ermilov et al. (2015a, b); the identification keys to all species of *G.* (*Cosmogalumna*) and *G.* (*Neogalumna*) were presented by Ermilov and Corpuz-Raros (2015) and Ermilov and Anichkin (2014b), respectively.

Material and methods

Exact collection locality and habitat are given in the respective "Material examined" section for each species.

Specimens were mounted in lactic acid on temporary cavity slides for measurement and illustration. The body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate. Notogastral width refers to the maximum width in dorsal aspect. Lengths of body setae were measured in lateral aspect. All body measurements are presented in micrometers. Formulas for leg setation are given in parentheses according to the sequence trochanter–femur–genu–tibia–tarsus (famulus included). Formulas for leg solenidia are given in square brackets according to the sequence genu–tibia–tarsus. General terminology used in this paper follows that of Grandjean (summarized by Norton and Behan-Pelletier 2009). Drawings were made with a camera lucida using a Carl Zeiss transmission light microscope "Axioskop-2 Plus".

Descriptions

Galumna (*Atypicogalumna*) subgen. n.

http://zoobank.org/89548A86-BC87-4288-9C4B-39FE1E4AD445

Type species. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n.

Subgeneric diagnosis. With main traits of the genus *Galumna* (see Ermilov et al. 2013). Notogaster with both porose areas and sacculi. Lamellar and sublamellar lines parallel, curving backwards. Body surface without sculpture and ornamentation. Adanal lyrifissures located near to anal aperture. Legs tridactylous.

Etymology. The specific name "*Atypicogalumna*" refers to the presence of porose areas and sacculi on the notogaster that is unusual for Galumnidae.

Remarks. *Galumna* (*Atypicogalumna*) subgen. n. differs from all genera and subgenera of the family Galumnidae by the presence of porose areas and sacculi on the notogaster (vs. either porose areas or sacculi present).

Galumna (*Atypicogalumna*) *corpuzrarosae* sp. n.

http://zoobank.org/D22C0050-5B3E-4218-AB18-EC4E7C8B6107
Figs 1–9

Diagnosis. Body size: 332–365 × 232–265. Rostral and lamellar setae setiform. Interlamellar setae represented by alveoli. Bothridial setae clavate. Anterior notogastral margin developed. Four pairs of rounded porose areas and three pairs of sacculi on notogaster. Median pore and postanal porose area present.

Description. *Measurements.* Body length: 332 (holotype: male), 332–365 (nine paratypes: three females and six males); notogaster width: 232 (holotype), 232–265 (nine paratypes). Without sexual dimorphism.

Integument. Body color light brown. Body surface, pteromorphs, genital and anal plates punctate (visible in dissected specimens), subcapitular mentum smooth. Several short longitudinal striae present in basal part of prodorsum (postero-laterally to alveoli of interlamellar setae).

Prodorsum. Rostrum rounded. Lamellar (*L*) and sublamellar (*S*) lines distinct. Rostral setae (*ro*, 26–28) setiform, barbed. Lamellar setae (*le*, 12–16) thin, indistinctly barbed. Interlamellar setae (*in*) represented by alveoli. Bothridial setae (*bs*, 57–61) clavate, with long stalk and shorter head rounded and barbed distally. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (16–18 × 6).

Notogaster. Anterior notogastral margin developed. Dorsophragmata elongated longitudinally. Four pairs of porose areas rounded, with distinct margins: *Aa* (16–20) slightly larger than *A1*, *A2* and *A3* (all 12–16). Three pairs of sacculi with minute channels and small openings: *Sa* located antero-medially and nearly to *Aa*, *S2* – medially and distanced to *A2*, *S3* – medially and nearly to *A3*. Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore present in all specimens,
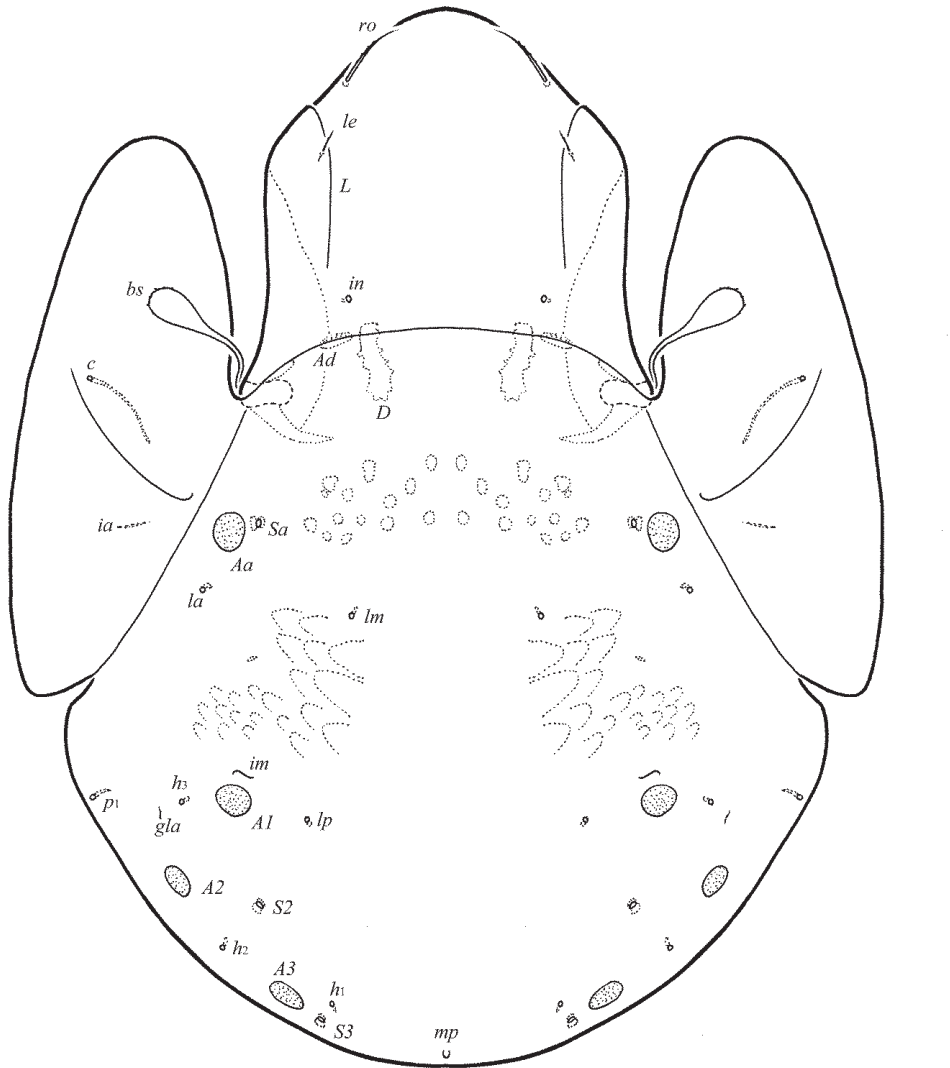
Figure 1. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n., adult: dorsal view. Scale bar 100 μm.

located between *A3*. All lyrifissures (*ia, im, ip, ih, ips*) distinct, *im* located anteriorly and nearly to *A1*. Opisthonotal gland openings located antero-laterally to *A2*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (see Engelbrecht 1969; Ermilov and Anichkin 2010). Subcapitulum size: 82–86 × 69–73. Subcapitular setae setiform, slightly barbed, *h* (6–8) shorter than *m* (10–12) and *a* (16), *a* thickest, *h* thinnest. Two pairs of adoral setae (*or*$_1$, *or*$_2$, 12) setiform, hook-like distally, barbed. Palps (53) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi (*sac*) distinct. Chelicerae (98) with two setiform, barbed setae; *cha* (34–36) longer than *chb* (22–24). Trägårdh's organ long, tapered.
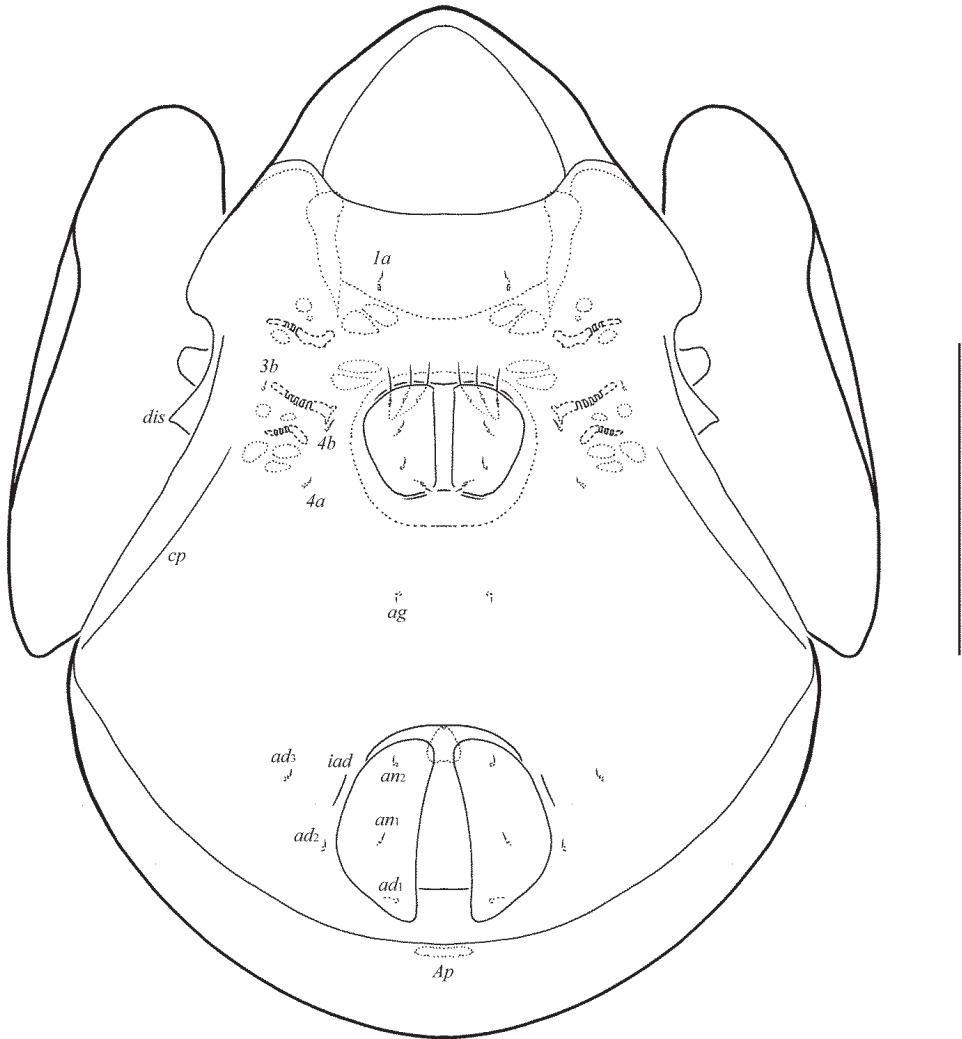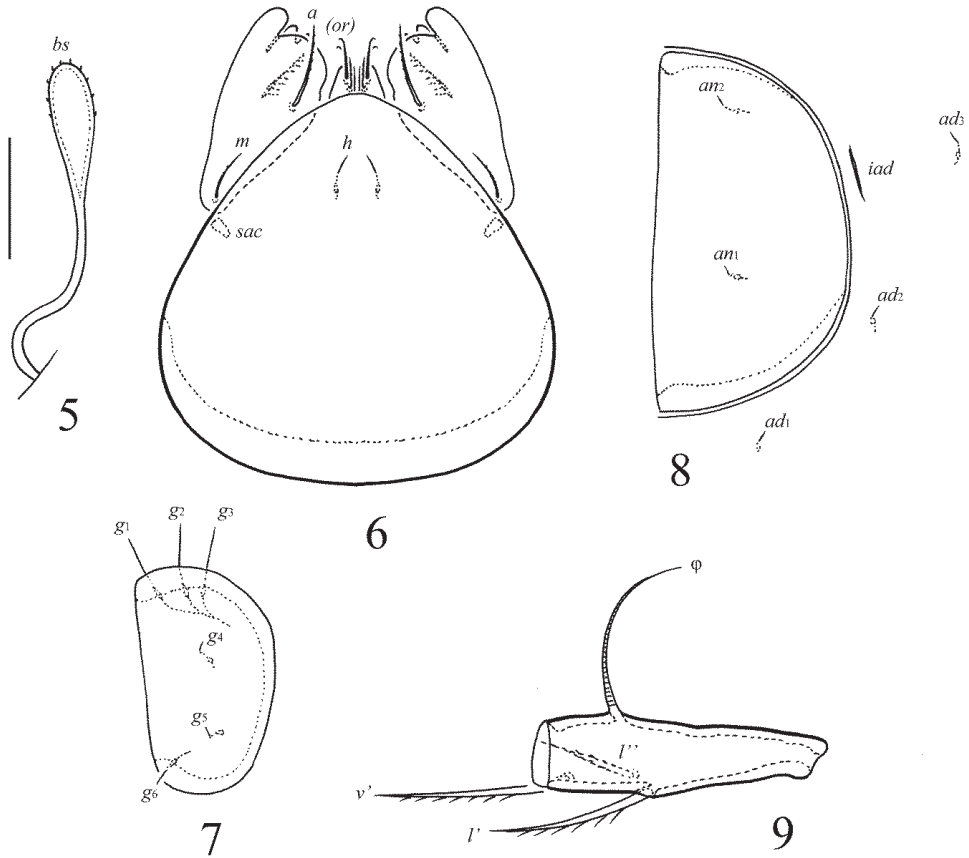
Figure 2. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–2. Setae (*1a*, *3b*, *4a*, *4b*) similar in length (4), thin, smooth. Pedotecta II rounded distally in ventral view. Discidia (*dis*) triangular. Circumpedal carinae (*cp*) distinct, clearly not reaching the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1–g_3, 8; g_4–g_6, 4), one pair of aggenital (*ag*, 4), two pairs of anal (*an_1*, *an_2*, 4) and three pairs of adanal (*ad_1*–*ad_3*, 4) setae thin, smooth. Three setae on anterior edge of each genital plate. Adanal setae distanced equal from each other, inserted in one diagonal row on each side of adanal region. Setae ad_3
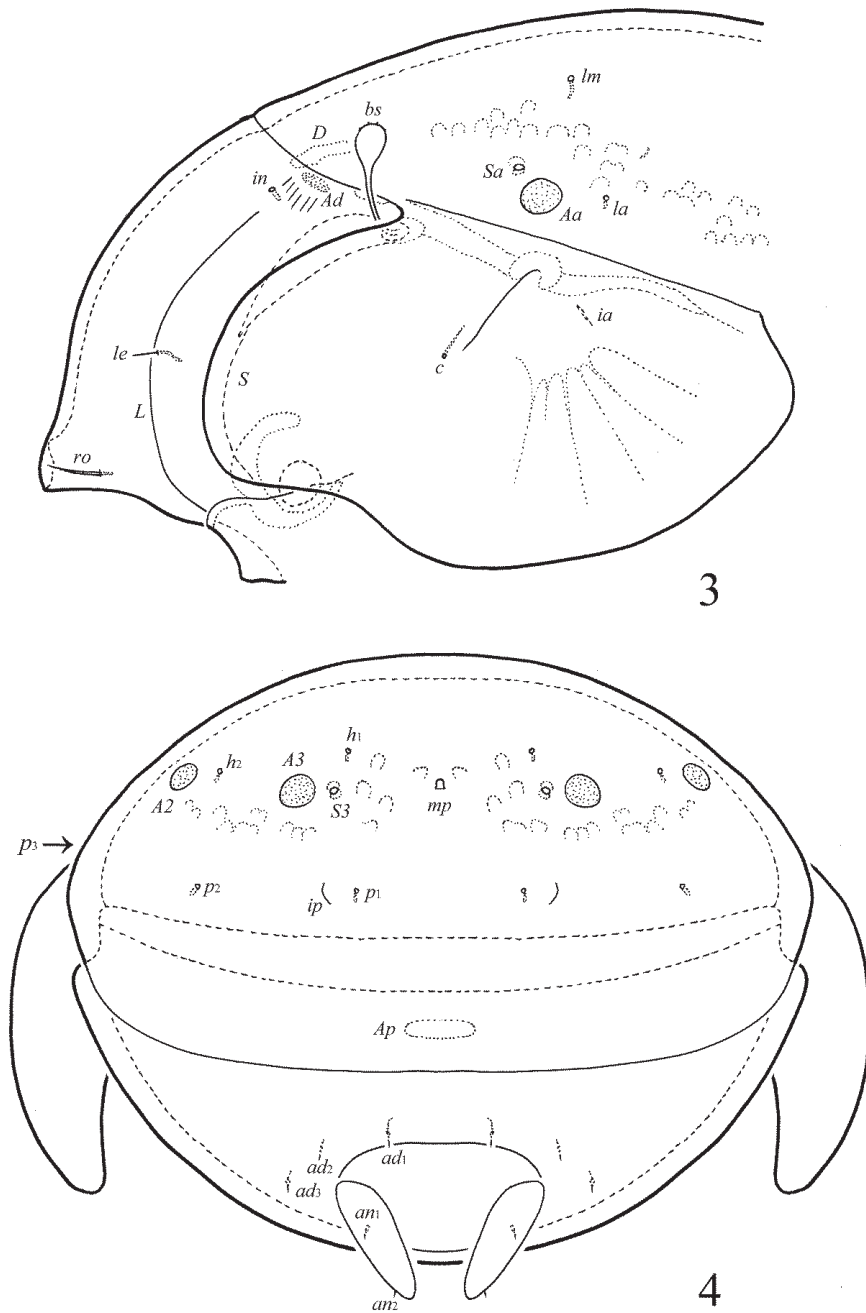
Figures 3–4. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n., adult: **3** anterior part of body, lateral view (gnathosoma and leg I not shown) **4** posterior view. Scale bar 100 μm.

Figures 5–9. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n., adult: **5** bothridial seta **6** subcapitulum, ventral view **7** genital plate, left **8** anal plate, left, and adanal setae **9** tibia of leg IV, right, antiaxial view. Scale bar 20 μm.

inserted laterally to adanal lyrifissures. Postanal porose area (*Ap*) elongate oval, transversely oriented (24 × 6).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (see Engelbrecht 1969; Ermilov and Anichkin 2010). Claws smooth. Formulas of leg setation and solenidia: I (1–4–3–4–20) [1–2–2], II (1–4–3–4–15) [1–1–2], III (1–2–1–3–15) [1–1–0], IV (1–2–2–3–12) [0–1–0]; homologies of setae and solenidia indicated in Table 1. Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.

Material examined. Holotype (male) and nine paratypes (three females and six males): Indonesia, Sumatra, Harapan landscape, jungle rubber agroforest, research site HJ1, 01°55'40.0"S, 103°15'33.8"E, 51 m a.s.l., in forest floor litter material. All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; six paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; three paratypes are deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Etymology. The specific name is dedicated to our friend and colleague, acarologist, Dr. Leonila Corpuz-Raros (Crop Protection Cluster, College of Agriculture and Museum of Natural History, University of the Philippines Los Baños, Los Baños, Philippines).

Remarks. *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. differs from the all species of the family Galumnidae by the presence of porose areas and sacculi on the notogaster (vs. either porose areas or sacculi in other species).

Galumna (Galumna) bidentatirostris sp. n.
http://zoobank.org/EBA76398-87BB-4A52-A76B-45CFE76940C7
Figs 10–19

Diagnosis. Body size: 564–664 × 448–514. Rostrum bidentate. Lamellar lines directed to lateral margins of prodorsum. Rostral setae curved medio-downwards. Lamellar setae shortest, interlamellar setae longest on prodorsum. Bothridial setae long, with dilated unilaterally, slightly barbed distally head. Anterior notogastral margin developed. Four pairs of oval porose areas on notogaster. Median pore and postanal porose area present.

Description. *Measurements.* Body length: 581 (holotype: male), 564–664 (five paratypes: two females and three males); notogaster width: 464 (holotype), 448–514 (five paratypes). Without sexual dimorphism.

Integument. Body color brown. Body surface, pteromorphs, subcapitular mentum, genital and anal plates punctate.

Prodorsum. Rostrum bidentate, teeth (*t*) strong. Lamellar and sublamellar lines distinct, curving backwards, slightly divergent in distal parts, lamellar lines directed to lateral margins of prodorsum. Rostral setae (41–49) indistinctly dilated basally and curved specifically medio-downwards, smooth. Lamellar setae (24–32) setiform, thin, slightly barbed. Interlamellar setae (69–73) setiform, straight, barbed. Bothridial setae (155–176) with long, smooth stalk and short, elongated, dilated unilaterally, slightly barbed distally head. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (12–16 × 4–6).

Notogaster. Anterior notogastral margin developed. Dorsophragmata elongated longitudinally. Four pairs of porose areas oval, with distinct margins: *Aa* (36–45 × 24–32) larger than *A1*, *A3* (32–36 × 20–24) and *A2* (16–24 × 12–20). Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore present in all specimens, located between *A2*. All lyrifissures distinct, *im* and opisthonotal gland openings located laterally to *A1*.
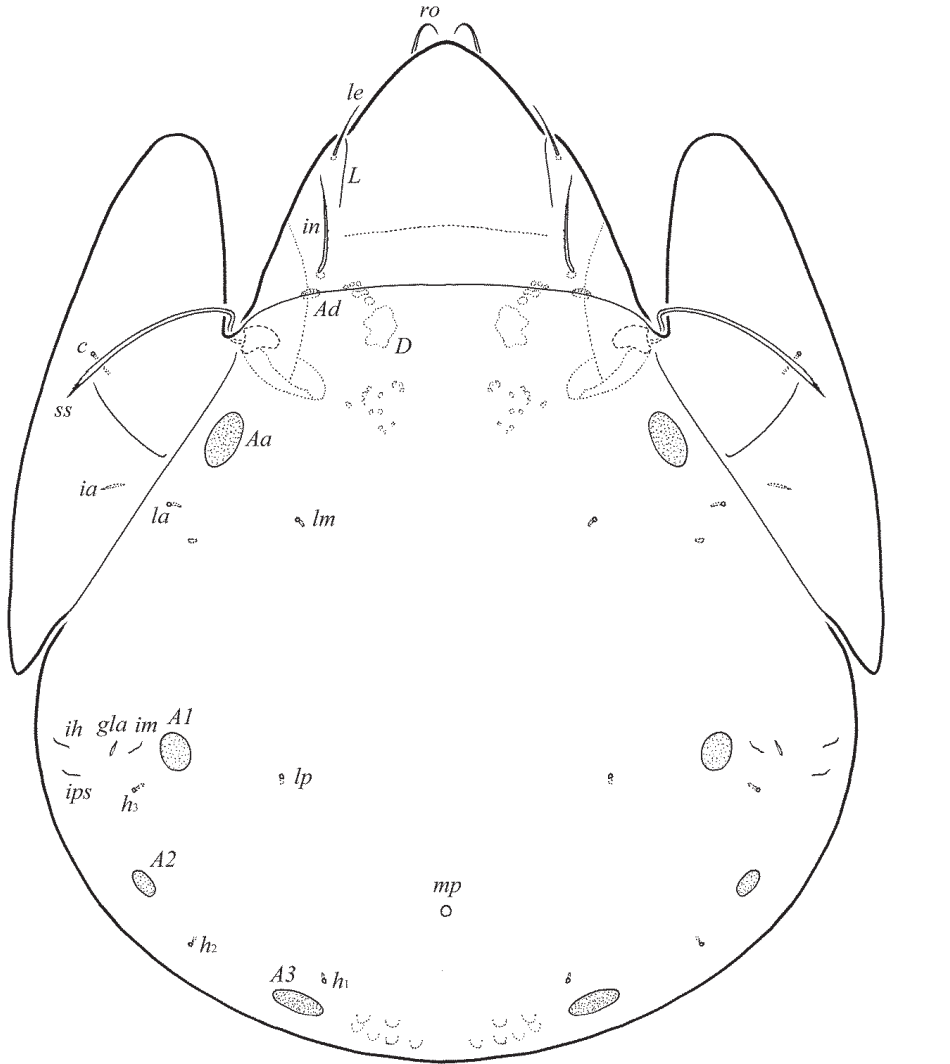
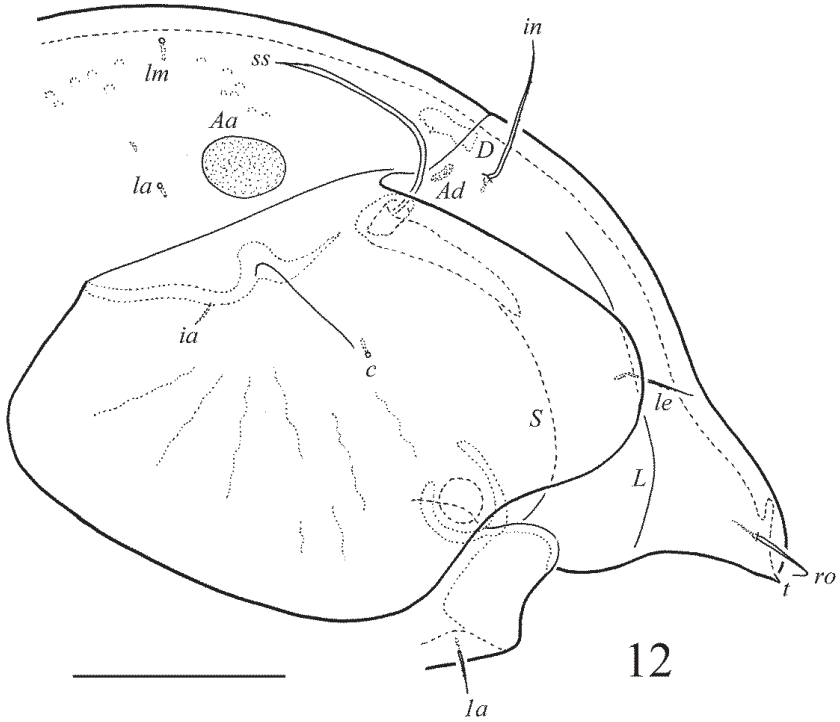**Figure 10.** *Galumna* (*Galumna*) *bidentatirostris* sp. n., adult: dorsal view. Scale bar 100 μm.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Subcapitulum size: 151–155 × 143–147. Subcapitular setae setiform, similar in thickness approximately, barbed, *h* (16) shorter than *m* (20–24) and *a* (24–28). Two pairs of adoral setae (16) setiform, hook-like distally, barbed. Palps (102–110) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (184–188) with two setiform, barbed setae; *cha* (65–69) longer than *chb* (41–45). Trägårdh's organ long, tapered.
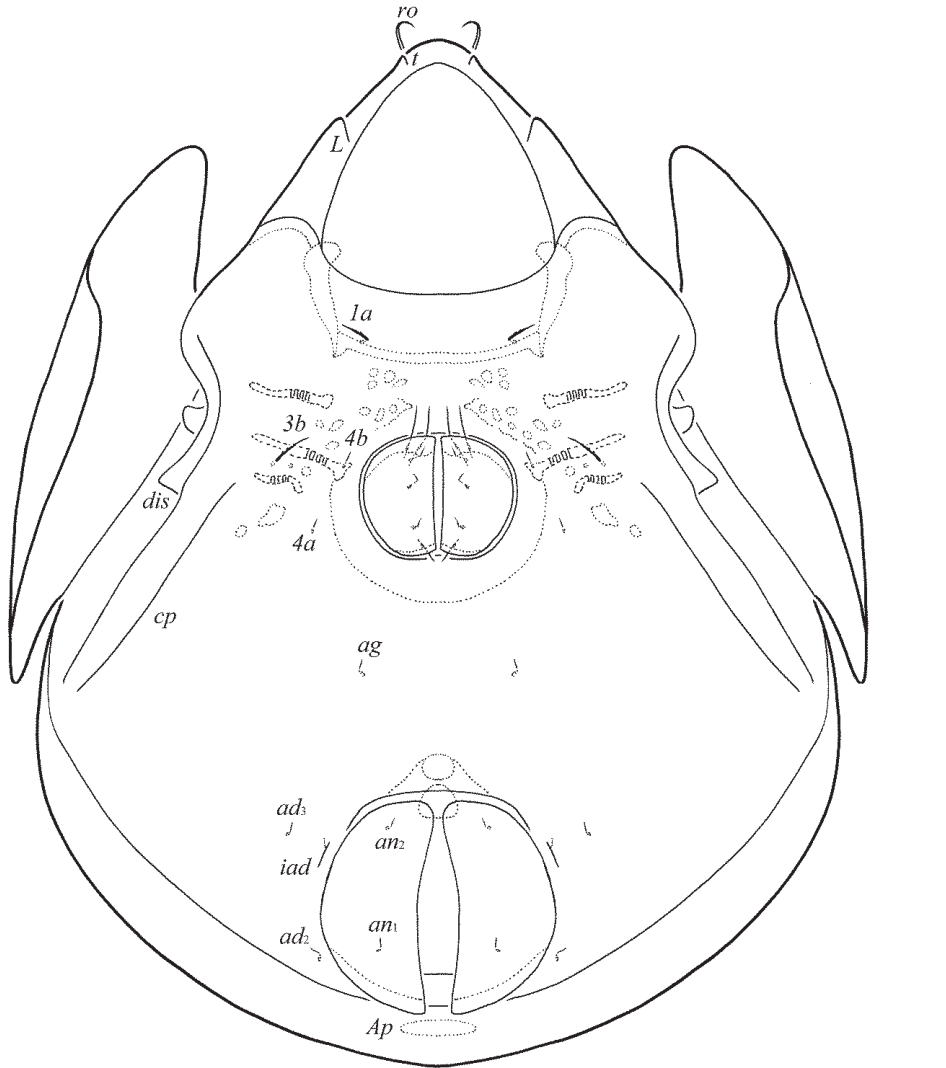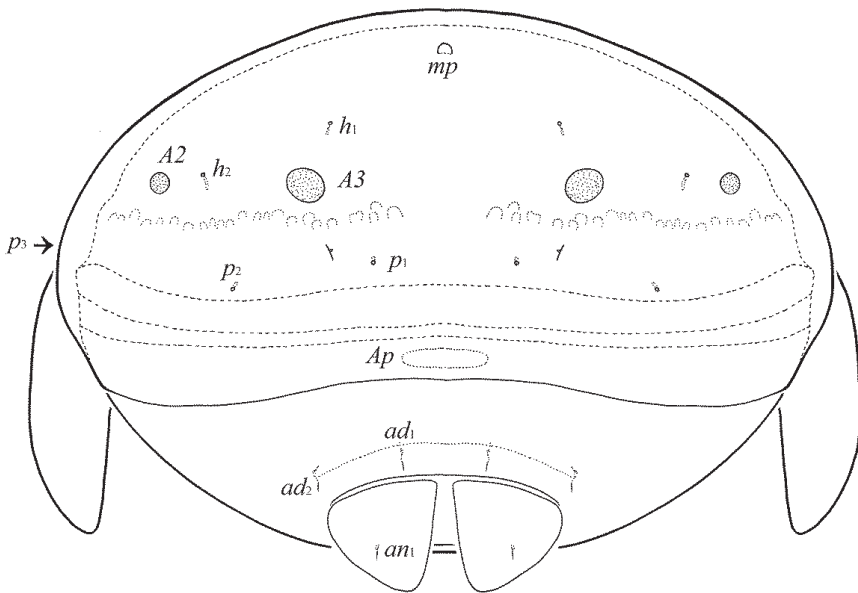
Figure 11. *Galumna* (*Galumna*) *bidentatirostris* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–2. Setae *1a* and *3b* (24–28) setiform, barbed; *4a* and *4b* (8) thin, smooth. Pedotecta II rounded anteriorly in ventral view. Discidia triangular. Circumpedal carinae distinct, little not reaching the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1, g_2, 14–18; g_3–g_6, 6–8), one pair of aggenital (8–12), two pairs of anal (8–12) and three pairs of adanal (8–12) setae thin, smooth. Two setae on anterior edge of each genital plate. Adanal setae ad_3 inserted
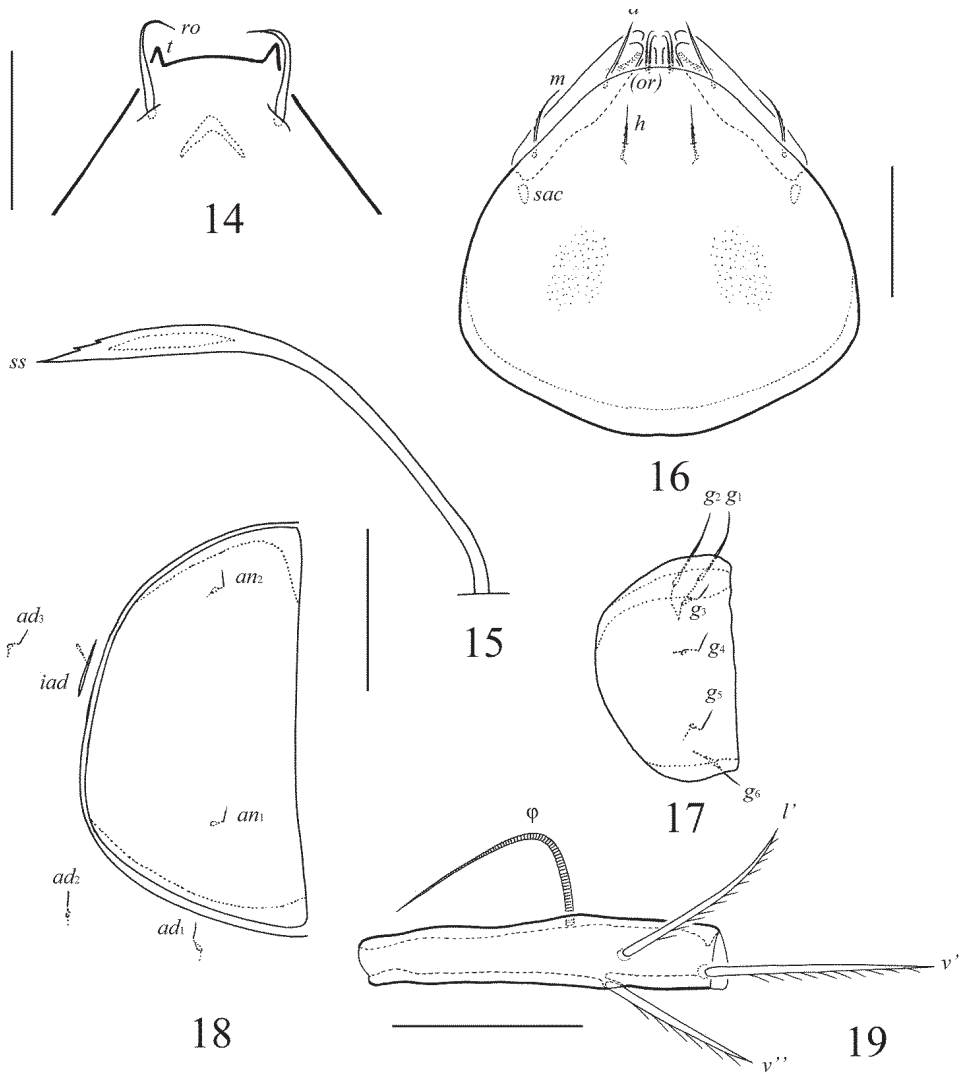
Figures 12–13. *Galumna* (*Galumna*) *bidentatirostris* sp. n., adult: **12** anterior part of body, lateral view (gnathosoma and leg I not shown) **13** posterior view. Scale bars 100 μm.

Figures 14–19. *Galumna* (*Galumna*) *bidentatirostris* sp. n., adult: **14** rostrum and rostral setae, dorso-frontal view **15** bothridial seta **16** subcapitulum, ventral view **17** genital plate, right **18** anal plate, right, and adanal setae **19** tibia of leg IV, left, antiaxial view. Scale bars 50 μm.

laterally to adanal lyrifissures. Postanal porose area elongated, transversally oriented (36–45 × 8–12).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.

Table 1. Leg setation and solenidia of adult *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (same data for *G.* (*Galumna*) *bidentatirostris* sp. n., *G.* (*G.*) *indonesica* sp. n., *G.* (*G.*) *mikoi* sp. n., *G.* (*Cosmogalumna*) *areticulata* sp. n., *G.* (*C.*) *sumatrensis* sp. n. and *G.* (*Neogalumna*) *specifica* sp. n.)

Leg	Tr	Fe	Ge	Ti	Ta
I	*v'*	*d*, (*l*), *bv"*	(*l*), *v'*, σ	(*l*), (*v*), φ_1, φ_2	(*ft*), (*tc*), (*it*), (*p*), (*u*), (*a*), *s*, (*pv*), *v'*, (*pl*), *l"*, ε, ω_1, ω_2
II	*v'*	*d*, (*l*), *bv"*	(*l*), *v'*, σ	(*l*), (*v*), φ	(*ft*), (*tc*), (*it*), (*p*), (*u*), (*a*), *s*, (*pv*), ω_1, ω_2
III	*v'*	*d*, *ev'*	*l'*, σ	*l'*, (*v*), φ	(*ft*), (*tc*), (*it*), (*p*), (*u*), (*a*), *s*, (*pv*)
IV	*v'*	*d*, *ev'*	*d*, *l'*	*l'*, (*v*), φ	*ft"*, (*tc*), (*p*), (*u*), (*a*), *s*, (*pv*)

Note: Roman letters refer to normal setae, Greek letters to solenidia (except ε = famulus). Single prime (') marks setae on the anterior and double prime (") setae on the posterior side of a given leg segment. Parentheses refer to a pair of setae. Tr – trochanter, Fe – femur, Ge – genu, Ti – Tibia, Ta – tarsus.

Material examined. Holotype (male) and five paratypes (two females and three males): Indonesia, Sumatra, Harapan landscape, oil palm plantation, research site HO1, 01°54'35.6"S, 103°15'58.3"E, 81 m a.s.l., in upper soil layer (0–5 cm). All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; two paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; three paratypes are deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Etymology. The specific name *bidentatirostris* refers to the bidentate rostrum.

Remarks. *Galumna* (*Galumna*) *bidentatirostris* sp. n. is morphologically most similar to *G.* (*G.*) *gibbula* Grandjean, 1956 from the Mediterranean (see Grandjean 1956) in having four pairs of oval porose areas on notogaster, long bothridial setae with elongated, unilaterally head, similar relative lengths of prodorsal setae (*in > ro > le*), median pore and elongated postanal porose area. However, the new species differs from the latter by the presence of bidentate rostrum (vs. without teeth in *G.* (*G.*) *gibbula*), rostral setae curved medio-backwards (vs. not curved in *G.* (*G.*) *gibbula*), anterior notogastral margin (vs. not developed in *G.* (*G.*) *gibbula*), lamellar lines directed to lateral margins of prodorsum (vs. directed to insertions of rostral setae), and the absence of an apophysis in the posterior part of the notogaster (vs. present in *G.* (*G.*) *gibbula*).

Galumna (*Galumna*) *indonesica* sp. n.

http://zoobank.org/508FCA63-79EB-4F0D-B909-7BD1A2EBF53A

Figs 20–28

Diagnosis. Body size: 498–531 × 365–381. Lamellar lines straight, directed to rostrum. Prodorsal setae setiform, barbed, lamellar setae shortest, interlamellar setae longest. Bothridial setae setiform, ciliate unilaterally. Anterior notogastral margin developed. Four pairs of porose areas present on notogaster, *Aa* booth-shaped to elongate triangular, transversally oriented, *A1*, *A2* and *A3* rounded. Median pore and postanal porose area present.

Figure 20. *Galumna* (*Galumna*) *indonesica* sp. n., adult: dorsal view. Scale bar 100 μm.

Description. *Measurements.* Body length: 531 (holotype: female), 498–531 (three paratypes: two females and one male); notogaster width: 381 (holotype), 365–381 (three paratypes). Without sexual dimorphism.

Integument. Body color brown. Body surface, pteromorphs, subcapitular mentum, genital and anal plates punctate (visible in dissected specimens).
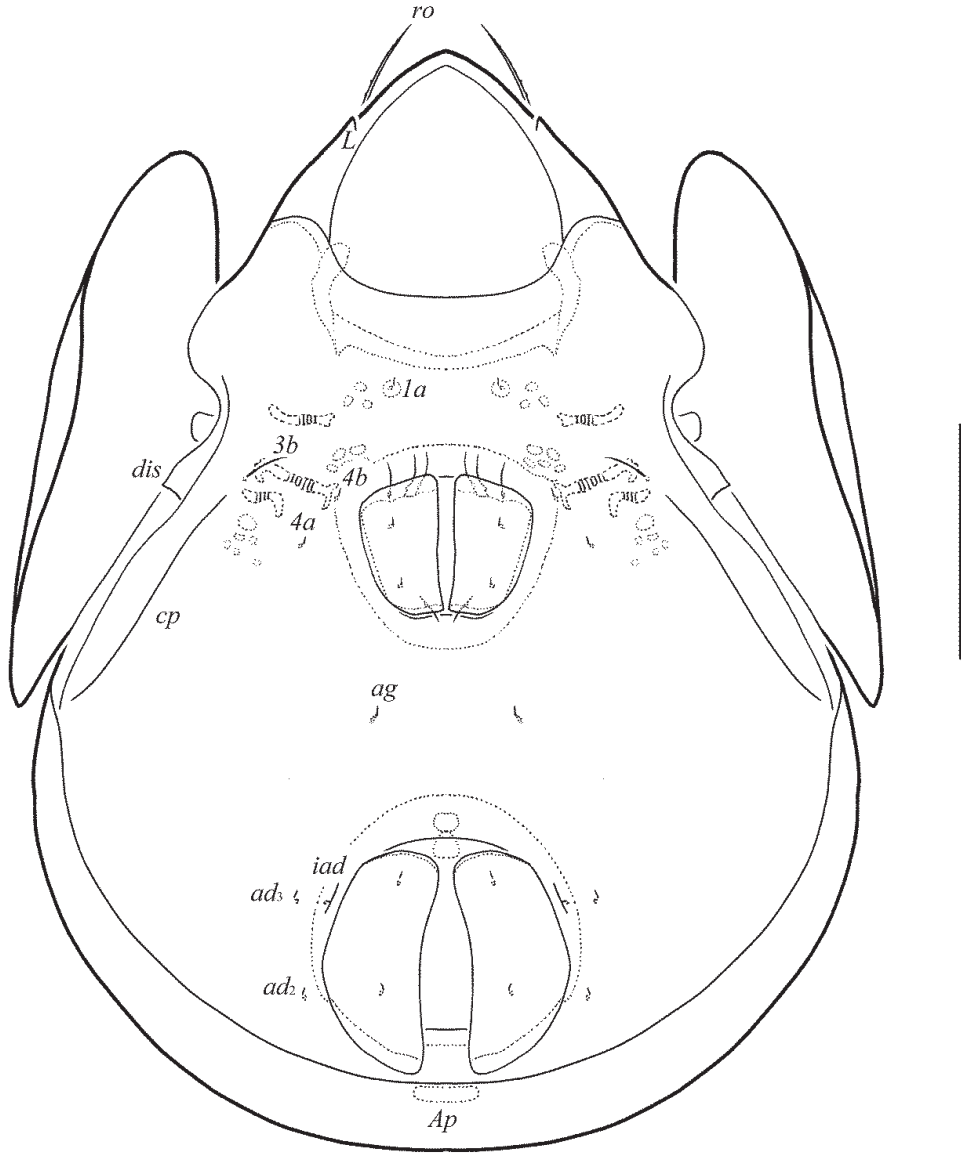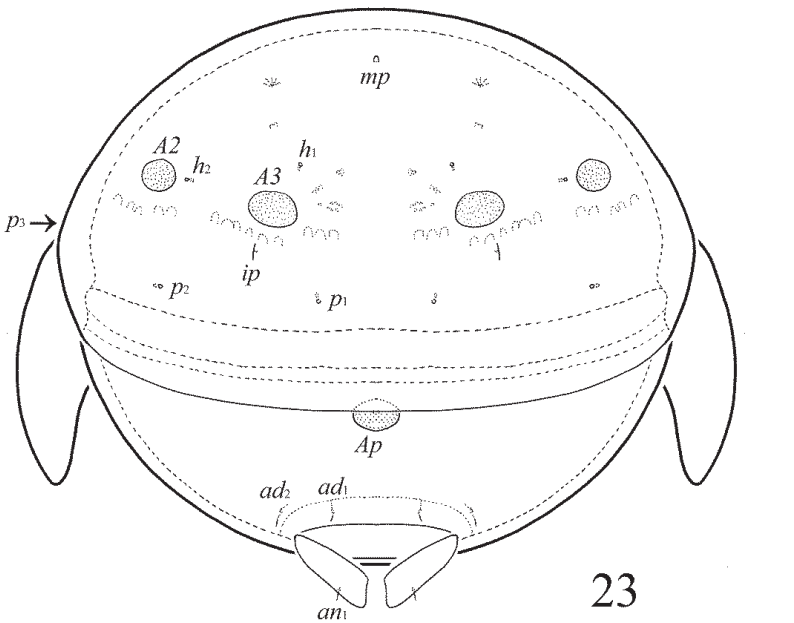
Figure 21. *Galumna* (*Galumna*) *indonesica* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

Prodorsum. Rostrum rounded. Lamellar lines straight, directed little laterally to insertions of rostral setae. Sublamellar lines curving backwards. Rostral (45–49), lamellar (24–28) and interlamellar (61–73) setae setiform, barbed. Bothridial setae (106–110) long, setiform, densely ciliate unilaterally. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (16–20 × 8–12).
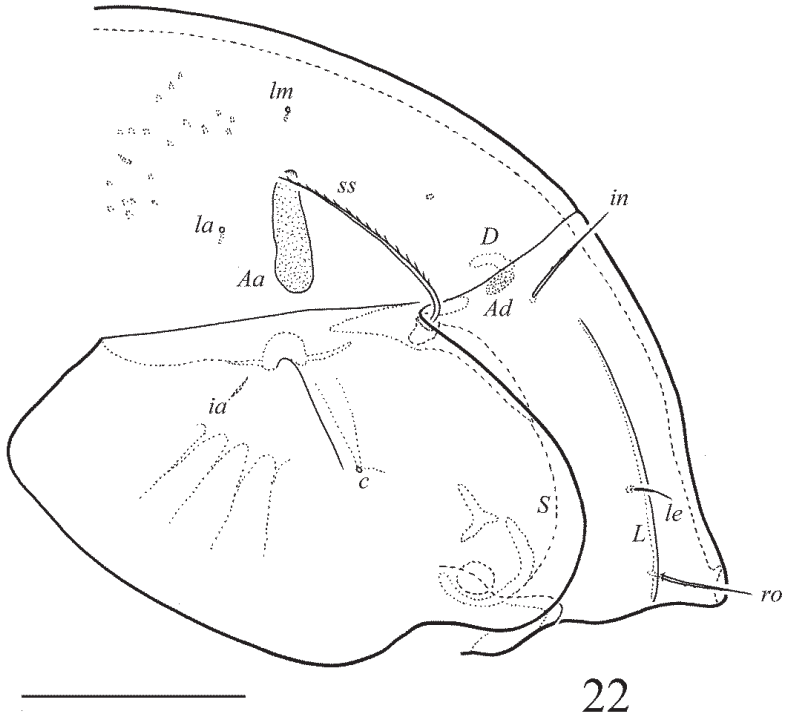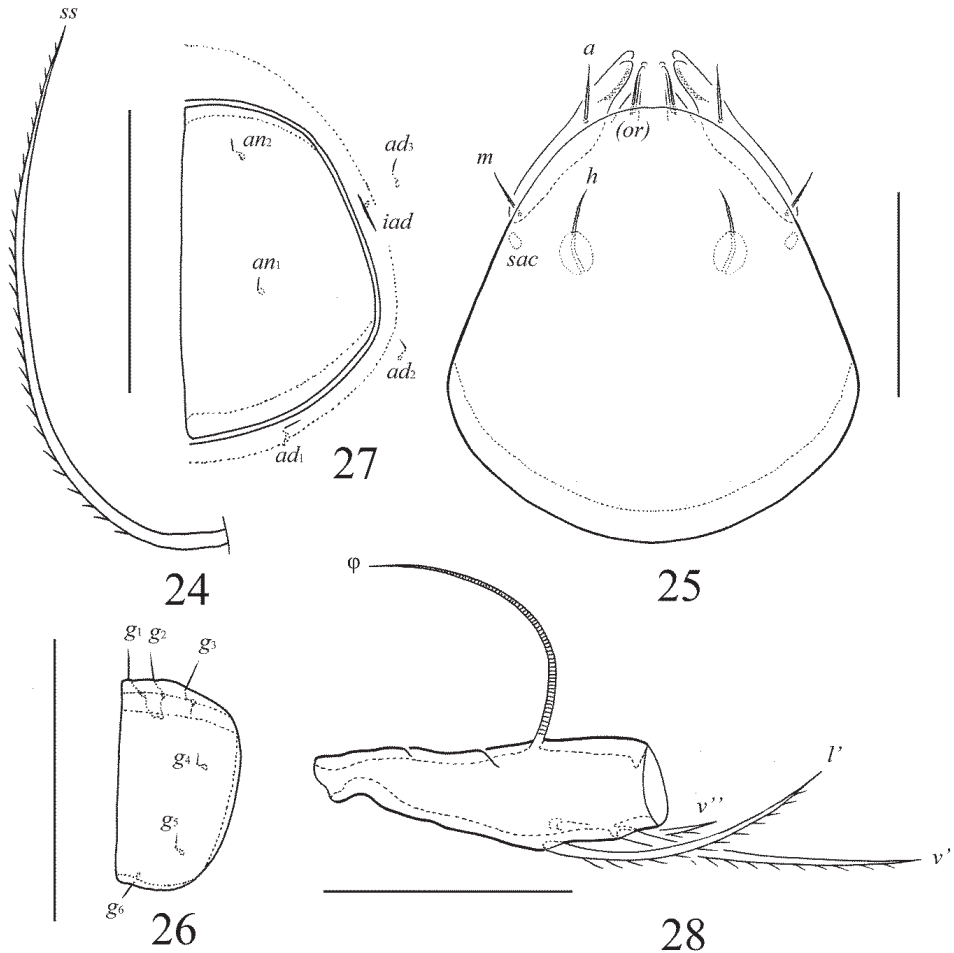
Figures 22–23. *Galumna* (*Galumna*) *indonesica* sp. n., adult: **22** anterior part of body, lateral view (gnathosoma and leg I not shown) **23** posterior view. Scale bars 100 μm.

Figures 24–28. *Galumna* (*Galumna*) *indonesica* sp. n., adult: **24** bothridial seta **25** subcapitulum, ventral view **26** genital plate, left **27** anal plate, left, and adanal setae **28** tibia of leg IV, left, antiaxial view. Scale bars 50 μm.

Notogaster. Anterior notogastral margin developed. Dorsophragmata elongated longitudinally. Four pairs of porose areas with distinct margins: *Aa* (36–49 × 12–16) booth-shaped to elongate triangular, transversally oriented; *A1*, *A2* and *A3* (24–32) rounded. Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore present in all specimens, located between *A2*. All lyrifissures distinct, *im* and opisthonotal gland openings located laterally to *A1*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Subcapitulum size: 118–123 × 102–106. Subcapitular setae setiform, similar in thickness approximately, barbed, *h* (18–20), *m* (20) and *a* (20–24) differ little in length. Two pairs of

adoral setae (16–18) setiform, hook-like distally, barbed. Palps (82) with typical seta-tion: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (155) with two setiform, barbed setae; *cha* (57) longer than *chb* (32). Trägårdh's organ long, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–2. Setae thin, smooth, *3b* (20–24) longer than *1a*, *4a* and *4b* (8). Pedotecta II rounded anteriorly in ventral view. Discidia triangular. Circumpedal carinae distinct, little, not reaching the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1–g_3, 8–10; g_4–g_6, 4), one pair of aggenital (4), two pairs of anal (4) and three pairs of adanal (4) setae thin, smooth. Three setae on anterior edge of each genital plate. Adanal setae ad_3 inserted laterally to adanal lyri-fissures. Postanal porose area elongated, transversally oriented (32–36 × 10–16).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.

Material examined. Holotype (female) and three paratypes (two females and one male): Indonesia, Sumatra, Bukit Duabelas landscape, jungle rubber agroforest, research site BJ5, 02°08'35.6"S, 102°51'04.7"E, 51 m a.s.l., in upper soil layer (0–5 cm). All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; two paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; one paratype is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

**Etymology.** The specific name *indonesica* refers to the country of origin, Indonesia.

Remarks. *Galumna* (*Galumna*) *indonesica* sp. n. is morphologically most similar to *G.* (*G.*) *parakazakhstani* Ermilov & Anichkin, 2014 from Vietnam (see Ermilov and Anichkin 2014a) in having lamellar lines directed to the anterior part of the prodorsum, setiform and ciliate bothridial setae, four pairs of notogastral porose areas with *Aa* elongated and transversally oriented, a median pore and an elongated postanal porose area. However, the new species differs from the latter by the position of rostral setae (nearly to the lamellar lines vs. distanced in *G.* (*G.*) *parakazakhstani*), the length of rostral and lamellar setae (rostral setae longer vs. lamellar setae longer in *G.* (*G.*) *parakazakhstani*) and the presence of anterior notogastral margin (vs. absent in *G.* (*G.*) *parakazakhstani*).

Galumna (*Galumna*) *mikoi* sp. n.
http://zoobank.org/ABED7400-EDB6-4D8F-9667-AD3426677A24
Figs 29–37

Diagnosis. Body size: 258–287 × 184–204. Surface of anogenital region and medio-anterior part of notogaster foveolate, surface of subcapitular mentum, genital and anal
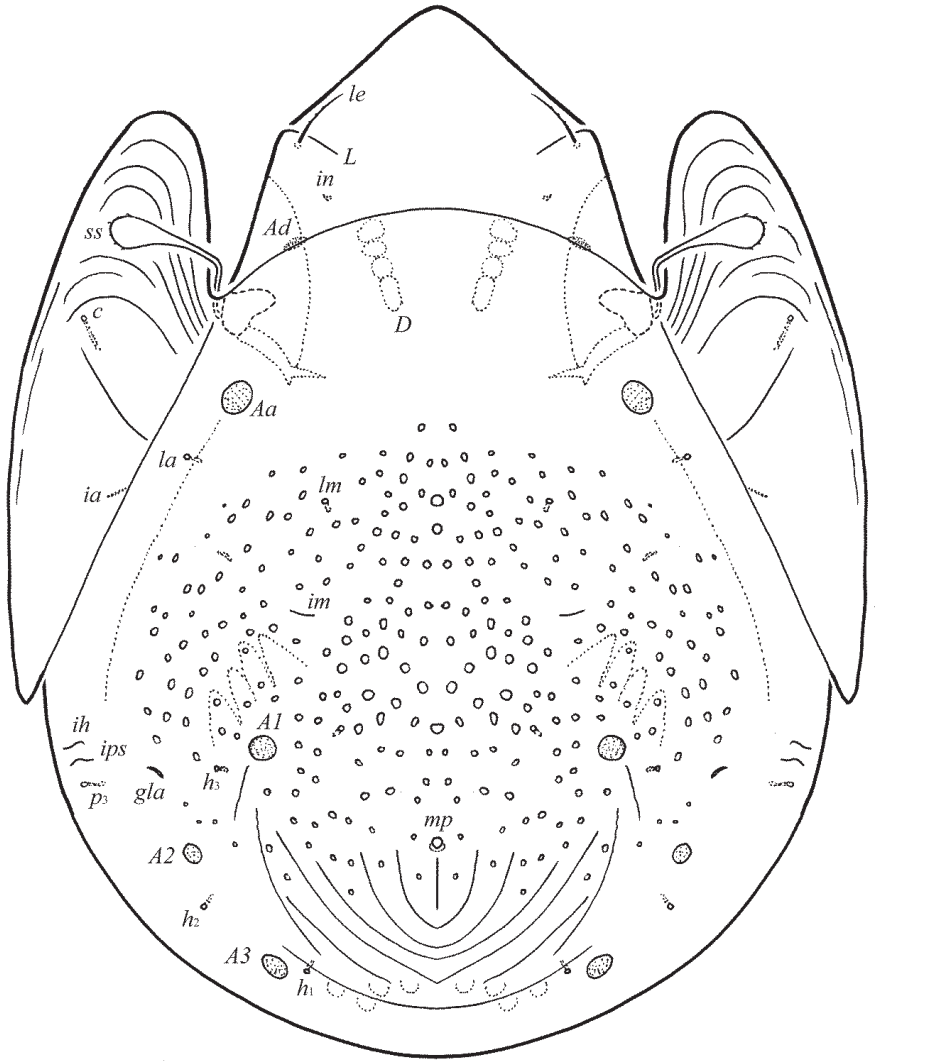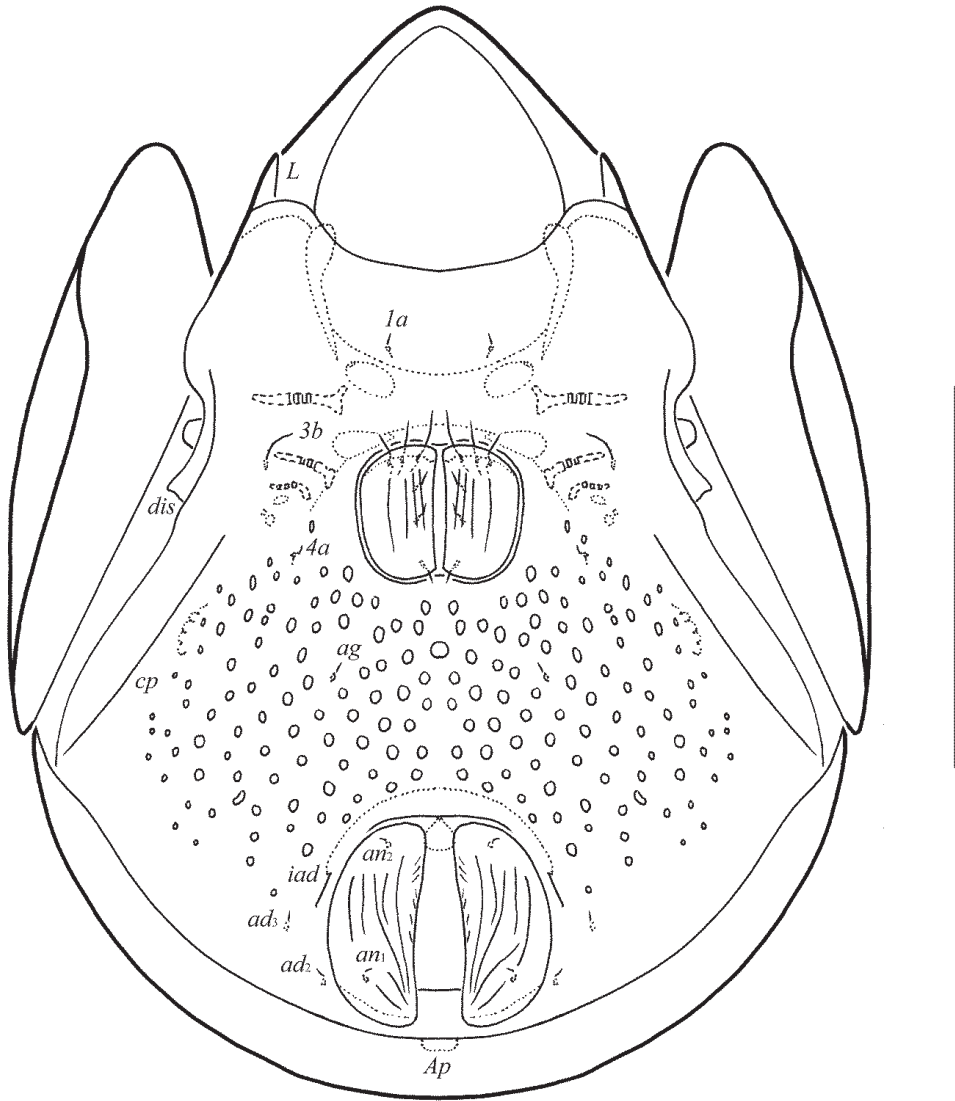
Figure 29. *Galumna* (*Galumna*) *mikoi* sp. n., adult: dorsal view. Scale bar 100 μm.

plates, antero-lateral parts of pteromorphs and posterior part of notogaster striate. Rostral and lamellar setae of medium size, interlamellar setae minute. Bothridial setae clavate. Anterior notogastral margin developed. Four pairs of rounded porose areas present on notogaster. Median pore and postanal porose area present.

Description. *Measurements.* Body length: 258 (holotype: male), 258–287 (three paratypes: two females and one male); notogaster width: 188 (holotype), 184–204 (three paratypes). Without sexual dimorphism.

Integument. Body color brown. Surface of anogenital region and medio-anterior part of notogaster foveolate (diameter of foveolae up to 6). Surface of subcapitular
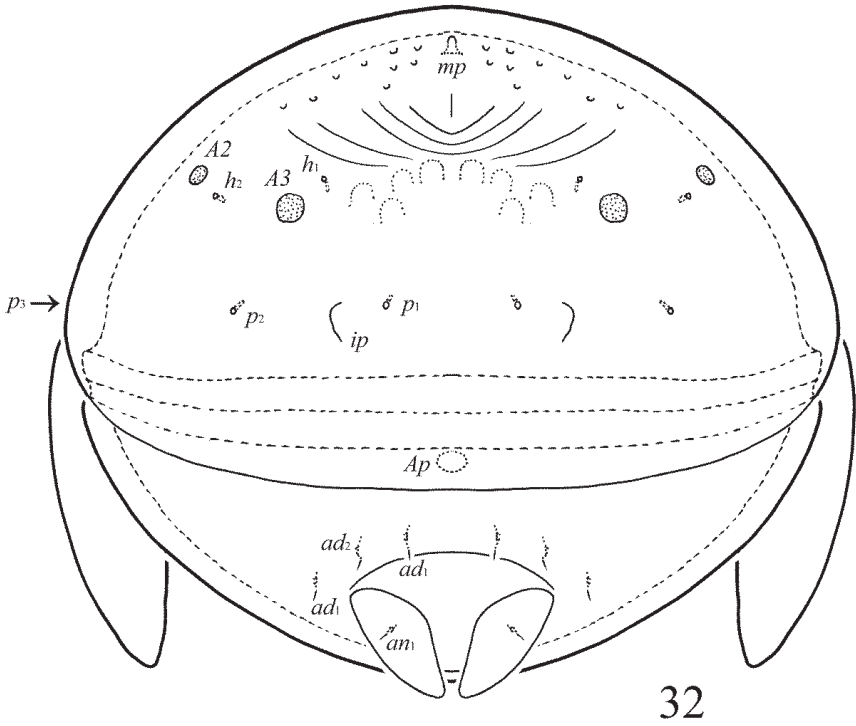
Figure 30. *Galumna* (*Galumna*) *mikoi* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

mentum, genital and anal plates, antero-lateral parts of pteromorphs and posterior part of notogaster striate.

Prodorsum. Rostrum rounded. Lamellar and sublamellar lines parallel, curving backwards. Rostral and lamellar setae similar in length (20–24), setiform, slightly barbed. Interlamellar setae minute (1). Bothridial setae (49–57) clavate, with long, smooth stalk and rounded, barbed head. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (6 × 4).
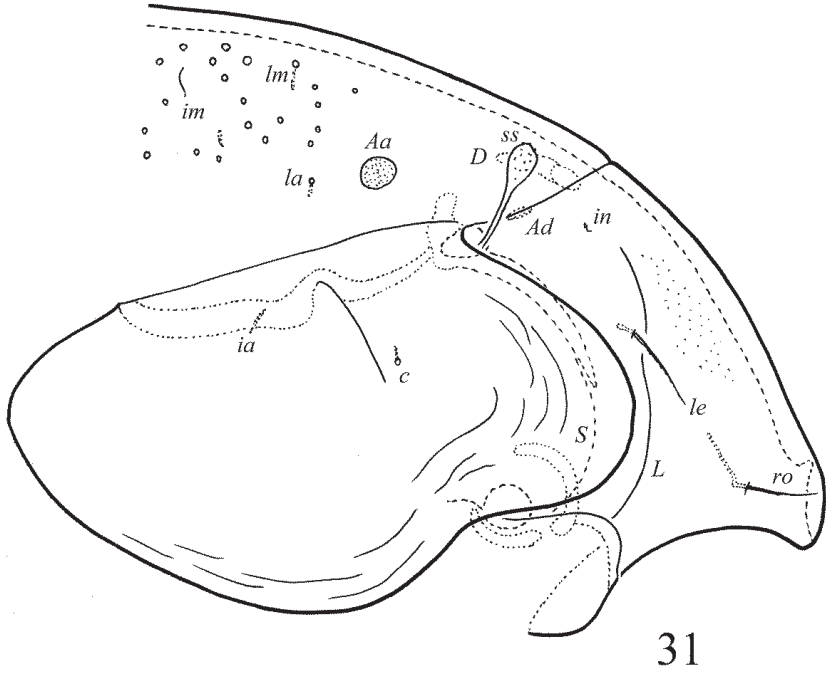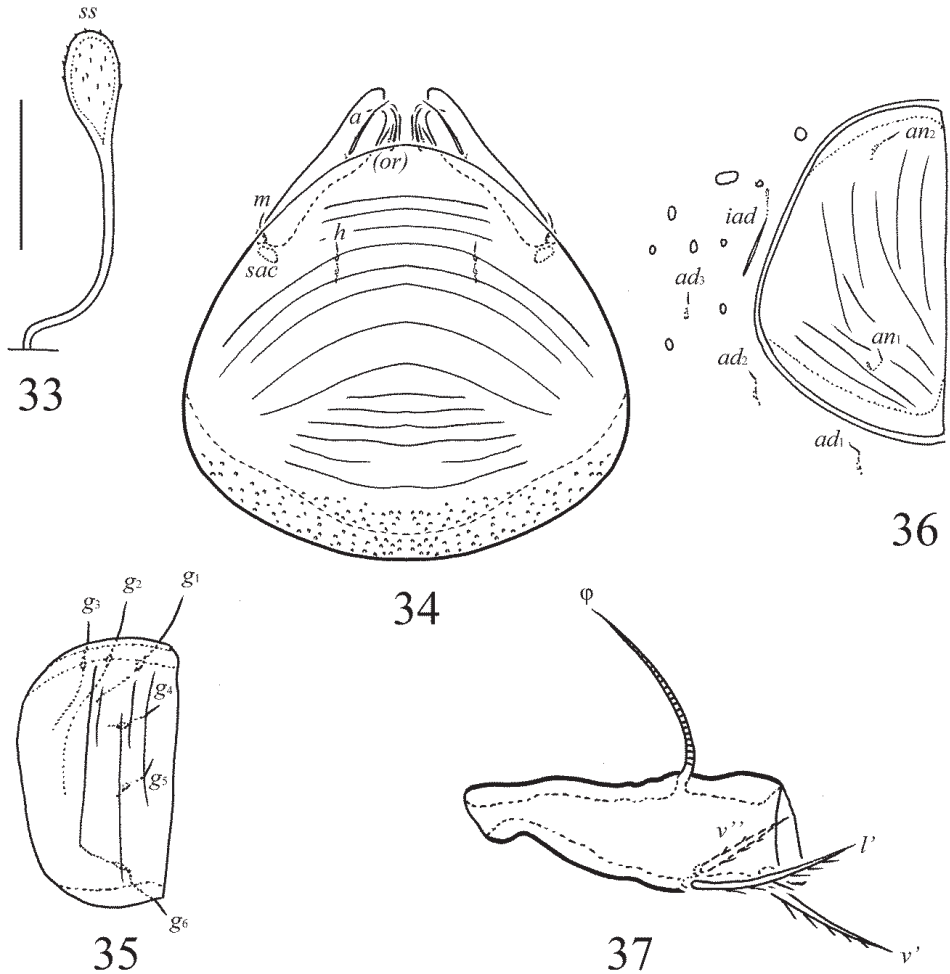
Figures 31–32. *Galumna* (*Galumna*) *mikoi* sp. n., adult: **31** anterior part of body, lateral view (gnathosoma and leg I not shown) **32** posterior view. Scale bar 100 μm.

Figures 33–37. *Galumna* (*Galumna*) *mikoi* sp. n., adult: **33** bothridial seta **34** subcapitulum, ventral view **35** genital plate, right **36** anal plate, right, and adanal setae **37** tibia of leg IV, left, antiaxial view. Scale bar 20 μm.

Notogaster. Anterior notogastral margin developed. Dorsophragmata elongated longitudinally. Four pairs of porose areas rounded, with distinct margins: *Aa* (8–12) larger than *A1*, *A2* and *A3* (6–8). Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore present in all specimens, located between *A2*. All lyrifissures distinct, *im* located between *lm* and *A1*. Opisthonotal gland openings located laterally to *A1*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Subcapitulum

size: 73–77 × 65–69. Subcapitular setae *a* (12–14) setiform, slightly barbed, longer and thicker than minute, smooth *m* and *h* (both pairs 4). Two pairs of adoral setae (8) setiform, hook-like distally, barbed. Palps (57) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (90) with two setiform, barbed setae; *cha* (32) longer than *chb* (20). Trägårdh's organ long, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–1. Setae thin, smooth, *3b* (8–10) longer than *1a* and *4a* (4). Pedotecta II rounded anteriorly in ventral view. Discidia triangular. Circumpedal carinae distinct, clearly not reach the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1, 8; g_2, 6; g_3–g_6, 4), one pair of aggenital (4), two pairs of anal (4) and three pairs of adanal (4) setae thin, smooth. Three setae on anterior edge of each genital plate. Adanal setae distanced equal from each other, inserted in one diagonal row on each side of adanal region. Adanal setae ad_3 inserted postero-laterally to adanal lyrifissures. Postanal porose area oval, transversally oriented (6–10 × 4–6).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Galumna*) (see Engelbrecht 1969; Ermilov and Anichkin 2010). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.

Material examined. Holotype (male): Indonesia, Sumatra, Harapan landscape, jungle rubber agroforest, research site HJ4, 01°47'07.3"S, 103°16'36.9"E, 57 m a.s.l., in forest floor litter material. Three paratypes (two females and one male): Indonesia, Sumatra, Harapan landscape, secondary rainforest, research site HF4, 02°11'15.2"S, 103°20'33.4"E, 77 m a.s.l., in forest floor litter material. All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; two paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; one paratype is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Etymology. The specific name is dedicated to our friend and colleague, acarologist, Dr. Ladislav Miko (Czech University of Life Sciences Prague, Charles University in Prague, Prague, Czech Republic).

Remarks. *Galumna* (*Galumna*) *mikoi* sp. n. is morphologically most similar to *G.* (*G.*) *innexa* Pérez-Íñigo & Baggio, 1986 from the Neotropical region (see Pérez-Íñigo and Baggio 1986) in having striate pteromorphs, minute interlamellar setae, anterior notogastral margin, four pairs of rounded porose areas on notogaster and median pore. However, the new species differs from the latter by the foveolate notogaster and anogenital region (vs. not foveolate in *G.* (*G.*) *innexa*), striate posterior part of notogaster (vs. not striate in *G.* (*G.*) *innexa*) and clavate bothridial setae (vs. lanceolate in *G.* (*G.*) *innexa*).

Galumna (*Cosmogalumna*) *areticulata* sp. n.
http://zoobank.org/3177798F-4D88-4538-9862-C51DD5495C85
Figs 38–46

Diagnosis. Body size: 298–315 × 215–249. Transverse band of strong, branched cerotegumental ridges developed in middle part of notogaster and between genital and anal plates, not forming a reticulate pattern, only a few cells present exeptionally. Rostral and lamellar setae short, interlamellar setae represented by alveoli. Bothridial setae clavate. Four pairs of rounded porose areas present on notogaster. Median pore and postanal porose area present.

Description. *Measurements.* Body length: 315 (holotype: male), 298–315 (seven paratypes: two females and five males); notogaster width: 249 (holotype), 215–249 (seven paratypes). Without sexual dimorphism.

Integument. Body color brown. Body surface, pteromorphs, genital and anal plates punctate (visible in dissected specimens). Subcapitular mentum smooth. Transverse band of strong, branched cerotegumental ridges developed in the middle part of the notogaster and between the genital and anal plates. These ridges comparatively short and not forming a clear reticulate pattern, only a few cells present exeptionally.

Prodorsum. Rostrum rounded. Lamellar and sublamellar lines parallel, curving backwards. Rostral setae (18–20) thin, smooth, pressed to the surface of prodorsum. Lamellar setae (6–8) minute. Interlamellar setae represented by alveoli. Bothridial setae (53–57) clavate, with long stalk and short head, rounded and smooth to slightly roughened distally. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (6–8 × 4–6).

Notogaster. Anterior notogastral margin developed. Dorsophragmata elongated longitudinally. Four pairs of porose areas rounded, with distinct margins: *Aa* (12–16) slightly larger than *A1*, *A2* and *A3* (all 8–10). Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore present in all specimens, located between *A2*. All lyrifissures distinct, *im* located between *lm* and *lp*. Opisthonotal gland openings located laterally to *A1*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Cosmogalumna*) (see Ermilov et al. 2011; Ermilov and Anichkin 2013). Subcapitulum size: 77–82 × 61–65. Subcapitular setae setiform, indistinctly barbed, *h* (4) shorter than *m* (6) and *a* (10–12), *a* thickest, *h* thinnest. Two pairs of adoral setae (6) setiform, hook-like distally, indistinctly barbed. Palps (69) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (94) with two setiform, barbed setae; *cha* (32) longer than *chb* (20). Trägårdh's organ long, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–2. Setae thin, smooth, *3b* (6) slightly longer than *1a*, *4a* and *4b* (4). Pedotecta II roundly triangular in ventral view. Discidia triangular. Circumpedal carinae distinct, clearly not reaching the insertions of setae *3b*.
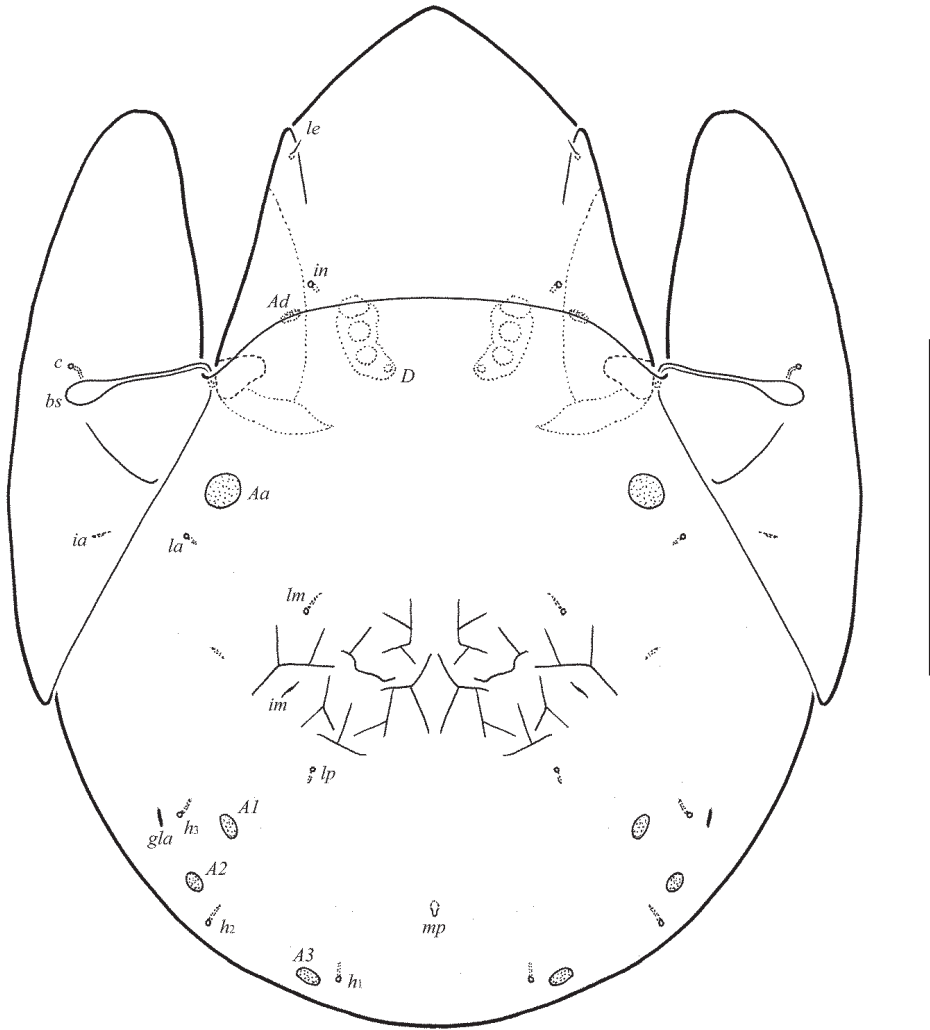
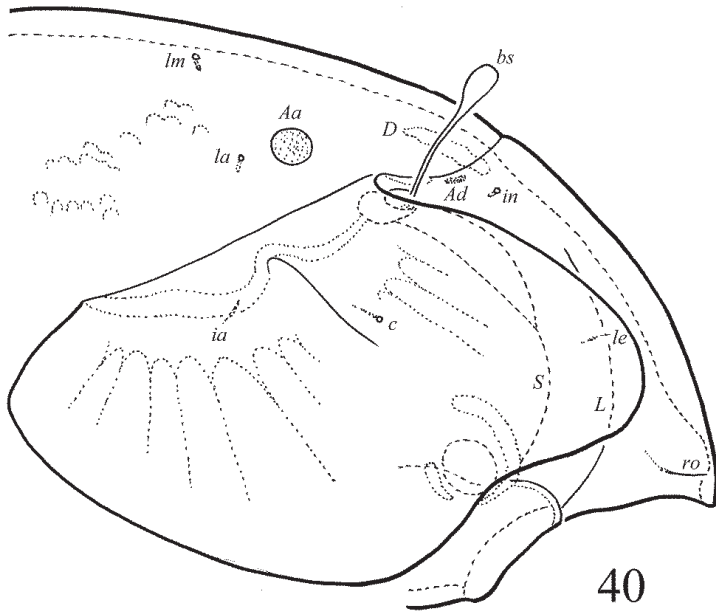**Figure 38.** *Galumna* (*Cosmogalumna*) *areticulata* sp. n., adult: dorsal view. Scale bar 100 µm.

Anogenital region. Six pairs of genital (g_1, g_2, 6–8; g_3–g_6, 4), one pair of aggenital (4), two pairs of anal (4) and three pairs of adanal (4) setae thin, smooth. Three setae on anterior edge of each genital plate. Adanal setae ad_3 inserted antero-laterally to adanal lyrifissures. Postanal porose area oval, transversally oriented (8–12 × 4).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Cosmogalumna*) (see Ermilov et al. 2011; Ermilov and Anichkin 2013). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.
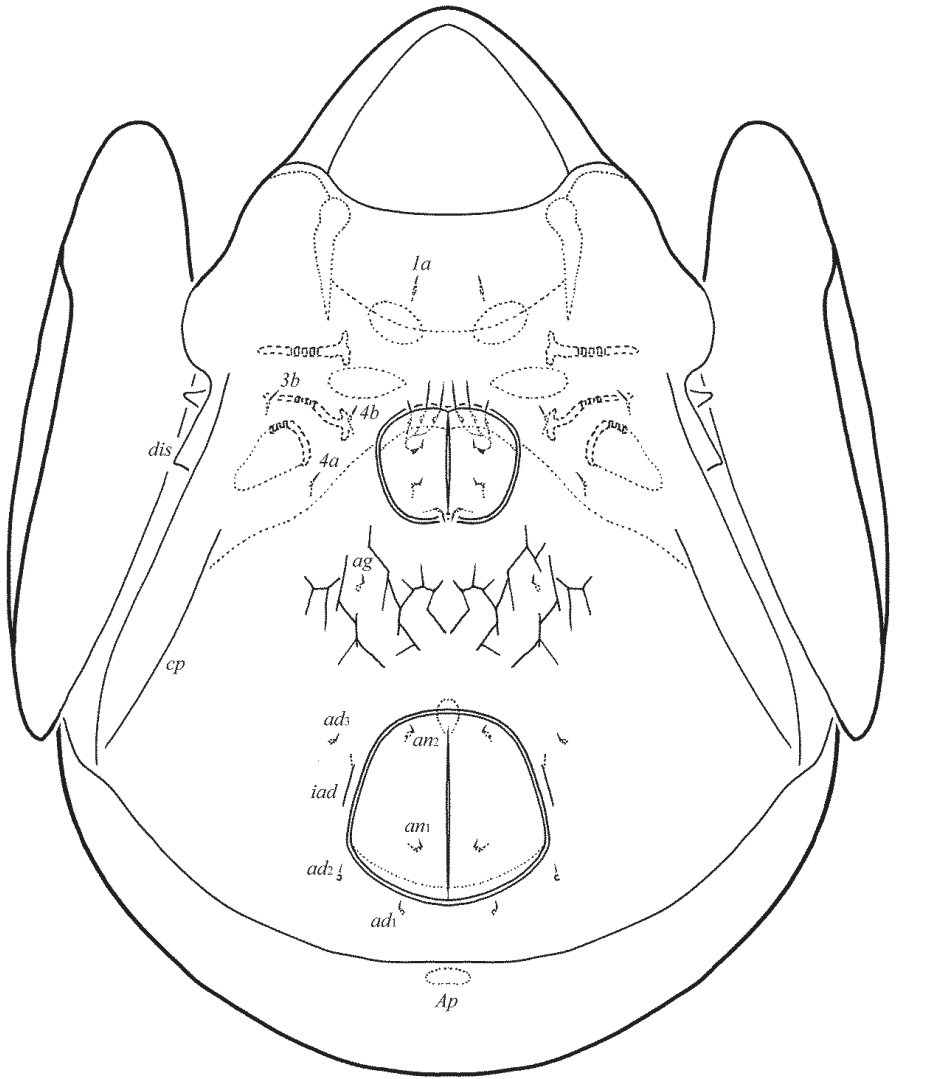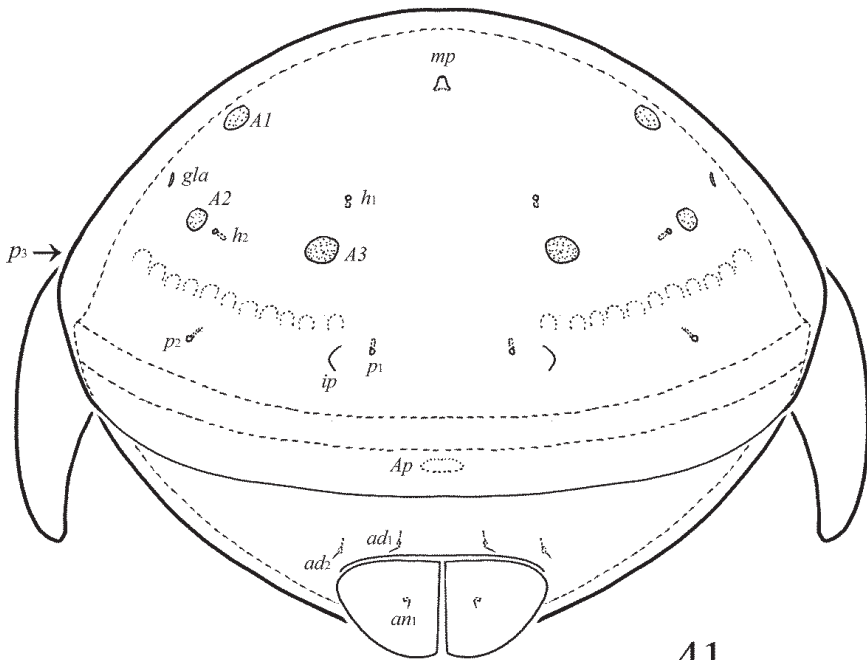
Figure 39. *Galumna* (*Cosmogalumna*) *areticulata* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

Material examined. Holotype (male) and two paratypes (one female and one male): Indonesia, Sumatra, Bukit Duabelas landscape, secondary rainforest, research site BF2, 01° 58'55.1"S, 102°45'02.7"E, 77 m a.s.l., in upper soil layer (0–5 cm). Five paratypes (one female and four males): Indonesia, Sumatra, Harapan landscape, jungle rubber agroforest, research site HJ1, 01°55'40.0"S, 103°15'33.8"E, 51 m a.s.l., in forest floor litter material. All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.
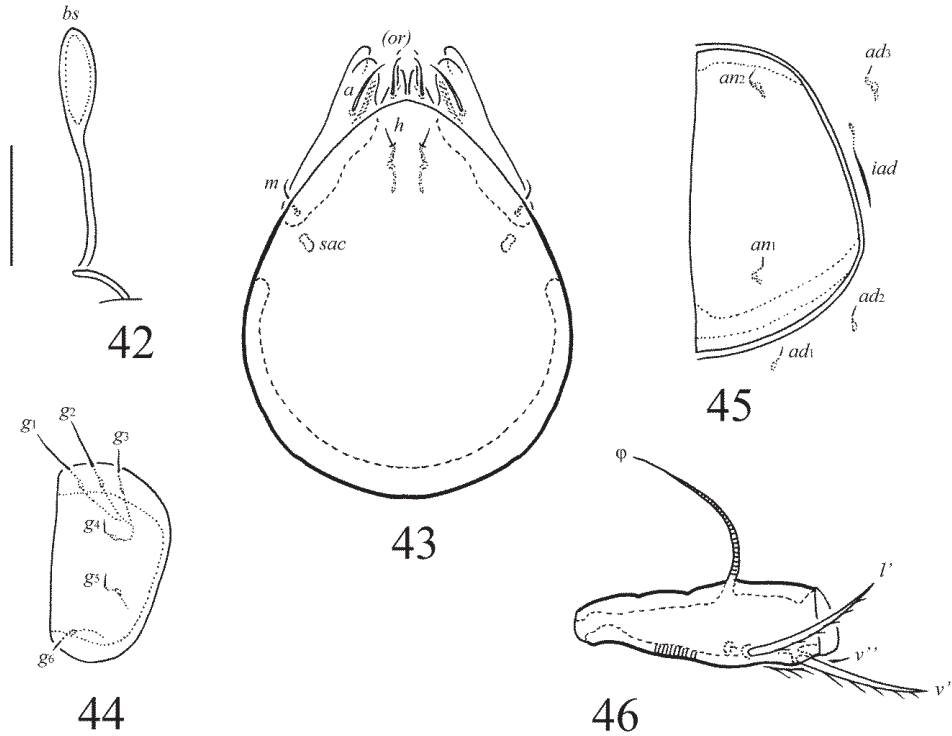
Figures 40–41. *Galumna* (*Cosmogalumna*) *areticulata* sp. n., adult: **40** anterior part of body, lateral view (gnathosoma and leg I not shown) **41** posterior view. Scale bar 100 μm.

Figures 42–46. *Galumna* (*Cosmogalumna*) *areticulata* sp. n., adult: **42** bothridial seta **43** subcapitulum, ventral view **44** genital plate, left **45** anal plate, left, and adanal setae **46** tibia of leg IV, left, antiaxial view. Scale bar 20 μm.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; two paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; six paratypes are deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Etymology. The specific name *areticulata* refers to the absence of clear reticulate pattern on the body.

Remarks. *Galumna* (*Cosmogalumna*) *areticulata* sp. n. is morphologically most similar to *G.* (*C.*) *praeoccupata* Subías, 2004 from southern China and Vietnam (see Aoki and Hu 1993; including personal data based on the Vietnamese specimens) in having transverse band of reticulation in the middle part of the notogaster and between genital and anal plates, and the absence of striate and reticulate pattern on the prodorsum and pteromorphs. However, the new species differs from the latter by the presence of strong, branched cerotegumental ridges, which do not form a reticulate pattern (vs. distinct reticulate pattern, represented by small, numerous, dense cells in *G.* (*C.*) *praeoccupata*), minute lamellar setae (vs. well developed in *G.* (*C.*) *praeoccupata*) and the directions of lamellar lines (to anterior tectum of ventral plate vs. to acetabula I in *G.* (*C.*) *praeoccupata*).

Galumna (*Cosmogalumna*) *sumatrensis* sp. n.

http://zoobank.org/D757C52A-EB98-4DE5-9D13-D3196FF12A7F

Figs 47–55

Diagnosis. Body size: 282–298 × 182–215. Reticulate pattern in the middle part of notogaster represented by few large cells, reticulate pattern between genital and anal plates represented by small, numerous, dense cells. Rostral and lamellar setae thin, indistinctly barbed, interlamellar setae represented by alveoli. Bothridial setae clavate. Four pairs of rounded porose areas on notogaster. Median pore absent. Postanal porose area present.

Description. *Measurements.* Body length: 282 (holotype: male), 282, 298 (two paratypes: one female and one male); notogaster width: 215 (holotype), 182, 215 (two paratypes). Without sexual dimorphism.

Integument. Body color brown. Body surface, pteromorphs, genital and anal plates, and subcapitular mentum punctate. Reticulate pattern in the middle part of notogaster present, cells large and not numerous. Reticulate pattern between genital and anal plates represented by small, numerous, dense cells.

Prodorsum. Rostrum rounded. Lamellar and sublamellar lines parallel, curving backwards. Rostral (16) and lamellar (10–12) setae thin, indistinctly barbed. Interlamellar setae represented by alveoli. Bothridial setae (49–53) clavate, with long stalk and short head, rounded and barbed distally. Exobothridial setae and their alveoli absent. Porose areas *Ad* oval, transversally oriented (14–16 × 4–6).

Notogaster. Anterior notogastral margin developed. Dorsophragmata large, elongated longitudinally. Four pairs of porose areas rounded, with distinct margins: *Aa* (14–16) larger than *A1*, *A2* and *A3* (all 8–10). Notogastral setae represented by 10 pairs of alveoli, *la* inserted posteriorly to *Aa*. Median pore absent in all specimens. All lyrifissures distinct, *im* located between *lm* and *A1*. Opisthonotal gland openings located antero-laterally to *A2*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Cosmogalumna*) (see Ermilov et al. 2011; Ermilov and Anichkin 2013). Subcapitulum size: 77 × 65–69. Subcapitular setae setiform, indistinctly barbed, *h* and *m* (all 6) shorter than *a* (12–14), *a* thickest, *h* thinnest. Two pairs of adoral setae (8) setiform, hook-like distally, indistinctly barbed. Palps (69) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (94) with two setiform, barbed setae; *cha* (32) longer than *chb* (20). Trägårdh's organ long, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–1. Setae *1a*, *3b* and *4a* similar in length (4), thin, smooth. Pedotecta II roundly triangular in ventral view. Discidia triangular. Circumpedal carinae distinct, clearly not reaching the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1, g_2, 8; g_3–g_6, 4), one pair of aggenital (4), two pairs of anal (4) and three pairs of adanal (4) setae thin, smooth. Three setae on anterior edge of each genital plate. Adanal setae ad_3 inserted laterally to adanal lyrifissures. Postanal porose area oval, transversally oriented (12–20 × 4–8).
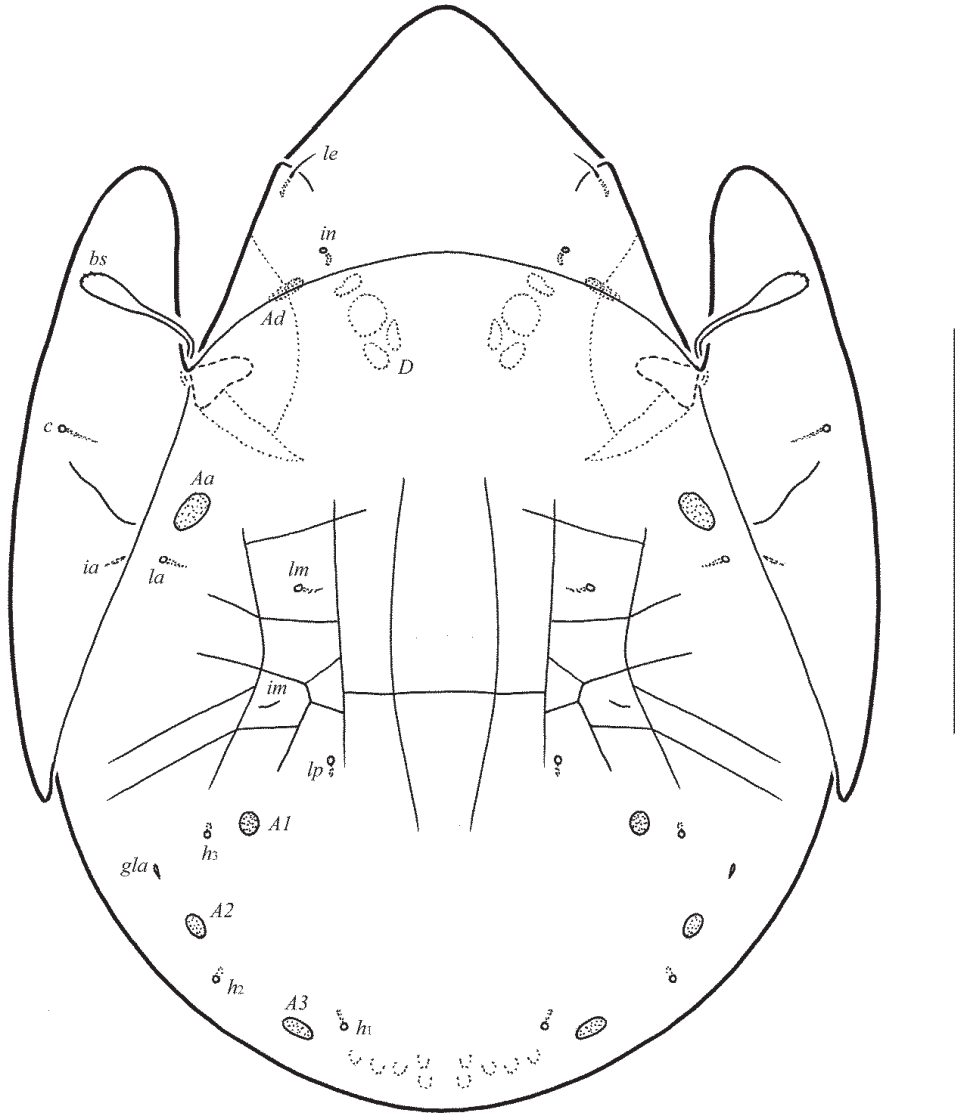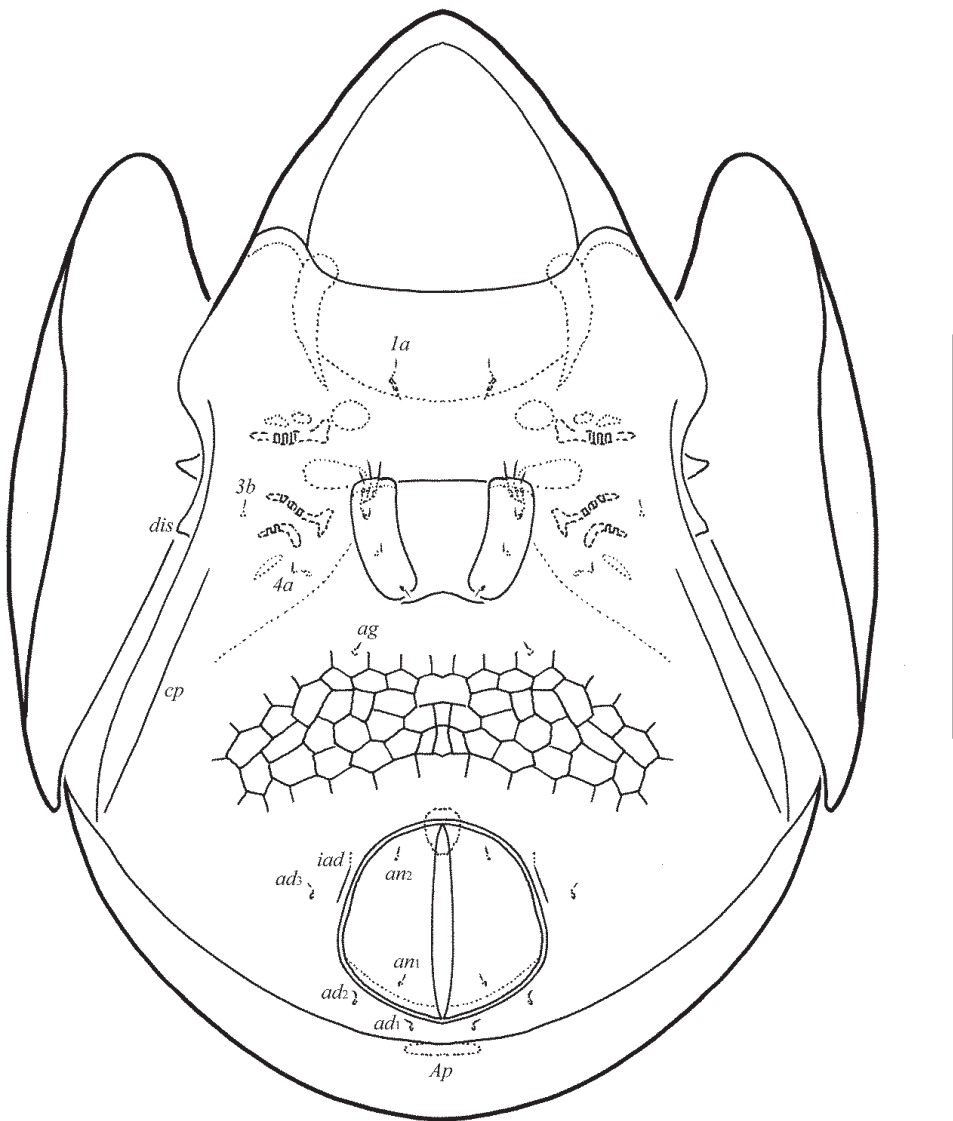
Figure 47. *Galumna* (*Cosmogalumna*) *sumatrensis* sp. n., adult: dorsal view. Scale bar 100 μm.

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Cosmogalumna*) (see Ermilov et al. 2011; Ermilov and Anichkin 2013). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.

Material examined. Holotype (male): Indonesia, Sumatra, Harapan landscape, secondary rainforest, research site HF4, 02°11'15.2"S, 103°20'33.4"E, 77 m a.s.l., in forest floor litter material. Two paratypes (one female and one male): Indonesia, Sumatra, Hara-
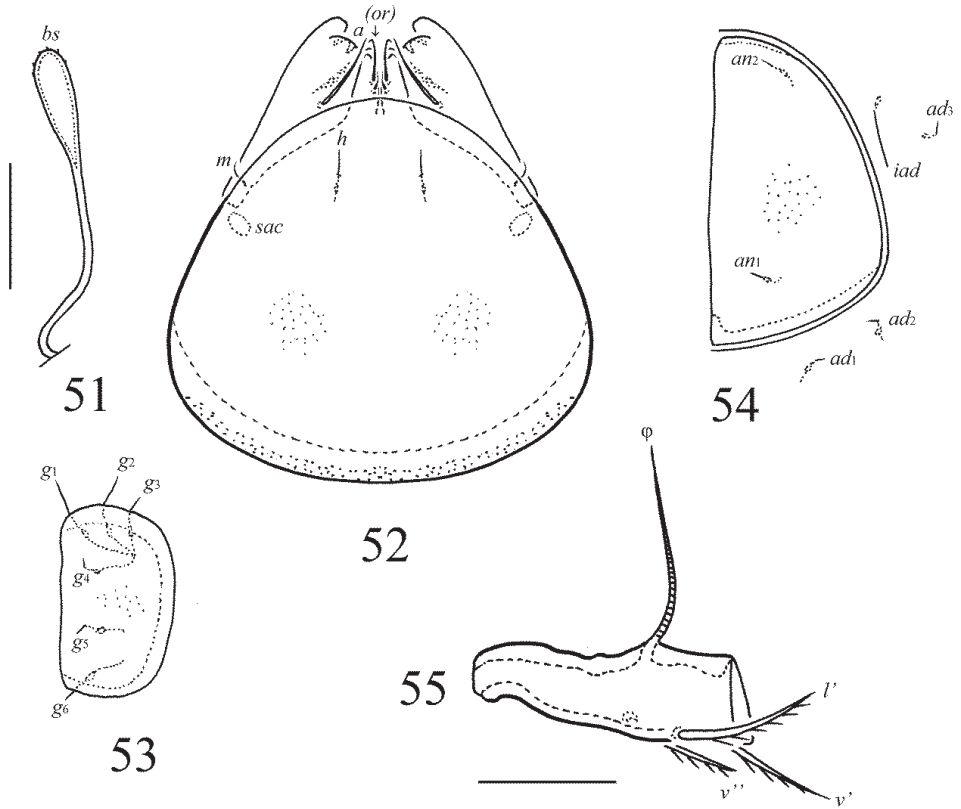
Figure 48. *Galumna* (*Cosmogalumna*) *sumatrensis* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

pan landscape, secondary rainforest, research site HF4, 02°11'15.2"S, 103°20'33.4"E, 77 m a.s.l., in upper soil layer (0–3 cm). All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; one paratype is deposited in the collection of the Senckenberg Museum, Görlitz, Germany; one paratype is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Figures 49–50. *Galumna* (*Cosmogalumna*) *sumatrensis* sp. n., adult: **49** anterior part of body, lateral view (gnathosoma and leg I not shown) **50** posterior view. Scale bar 100 μm.

Figures 51–55. *Galumna* (*Cosmogalumna*) *sumatrensis* sp. n., adult: **51** bothridial seta **52** subcapitulum, ventral view **53** genital plate, left **54** anal plate, left, and adanal setae **55** tibia of leg IV, left, antiaxial view. Scale bars 20 μm.

Etymology. The specific name *sumatrensis* refers to the island of origin, Sumatra.

Remarks. *Galumna* (*Cosmogalumna*) *sumatrensis* sp. n. is morphologically most similar to *G.* (*C.*) *praeoccupata* Subías, 2004 from southern China and Vietnam (see Aoki and Hu 1993; including personal data based on the Vietnamese specimens) and *G.* (*C.*) *areticulata* sp. n. from Indonesia in having a transverse band of reticulation in the middle part of the notogaster and between genital and anal plates, and the absence of striate and reticulate pattern on the prodorsum and pteromorphs. The new species differs from *G.* (*C.*) *praeoccupata* by the presence of large and not numerous of reticulate cells on notogaster (vs. pattern distinct, represented by small, numerous, dense cells in *G.* (*C.*) *praeoccupata*) and absence of median pore (vs. present in *G.* (*C.*) *praeoccupata*). The new species differs from *G.* (*C.*) *areticulata* sp. n. by the presence of reticulate pattern in the anogenital region represented by small, numerous, dense cells (vs. strong, branched cerotegumental ridges, which do not form a reticulate pattern present in *G.* (*C.*) *areticulata* sp. n.) and the absence a median pore (vs. present in *G.* (*C.*) *areticulata* sp. n.).

Galumna (*Neogalumna*) *specifica* sp. n.
http://zoobank.org/259FF377-30D3-4B95-8E74-12D956CC4F96
Figs 56–64

Diagnosis. Body size: 498–531 × 348–365. Subcapitular mentum, genital plates and basal part of prodorsum striate. Lamellar lines straight, divergent to sublamellar lines medio-anteriorly. Prodorsal setae setiform, barbed, *le* thinnest. Bothridial setae, setiform, ciliate. Anterior notogastral margin developed. Notogastral setal alveoli *la* absent, c_x present. Four pairs of rounded porose areas on notogaster. Median pore absent. Postanal porose area elongate oval.

Description. *Measurements.* Body length: 498 (holotype: male), 498–531 (three paratypes: one female and two males); notogaster width: 348 (holotype), 348–365 (three paratypes). Without sexual dimorphism.

Integument. Body color brown. Body surface, pteromorphs and anal plates smooth. Subcapitular mentum, genital plates and basal part of prodorsum with longitudinal striae.

Prodorsum. Rostrum rounded. Medio-anterior part slightly elongate, hump-like. Lamellar lines straight, directed to insertions of rostral setae, but clearly not reaching them. Sublamellar lines curving backwards. Rostral (39–45), lamellar (18–20) and interlamellar (18–20) setae setiform, barbed, lamellar setae thinnest. Bothridial setae long (106–114), setiform, unilaterally ciliate. Exobothridial setae and their alveoli absent. Porose areas *Ad* narrow, elongate oval, transversally oriented (28–34 × 4).

Notogaster. Anterior notogastral margin developed. Dorsophragmata of medium size, elongated longitudinally. Four pairs of porose areas rounded, with distinct margins: *Aa* (16–24) usually slightly larger than *A1*, *A2* and *A3* (all 12–16). Notogastral setae represented by 10 pairs of alveoli, however, based on their localization, *la* absent and c_x present. Median pore absent in all specimens. All lyrifissures distinct, *im* located between *lm* and *A1*. Opisthonotal gland openings located laterally to *A1*.

Gnathosoma. Morphology of subcapitulum, palps and chelicerae typical for *Galumna* (*Neogalumna*) (see Ermilov and Anichkin 2010, 2014b). Subcapitulum size: 110–114 × 98–102. Subcapitular setae setiform, indistinctly barbed, *h* (6–8) shorter than *a* and *m* (both pairs 12), *a* thickest, *h* thinnest. Two pairs of adoral setae (6–8) setiform, hook-like distally, indistinctly barbed. Palps (94) with typical setation: 0–2–1–3–9(+ω). Axillary sacculi distinct. Chelicerae (139) with two setiform, barbed setae; *cha* (45) longer than *chb* (24). Trägårdh's organ long, tapered.

Epimeral and lateral podosomal regions. Anterior tectum of epimere I smooth. Apodemes 1, 2, sejugal and 3 well visible. Setal formula: 1–0–1–2. Setae thin, smooth, *3b* (32–41) longer than *1a*, *4a* and *4b* (6–8). Pedotecta II distally rounded in ventral view. Discidia triangular. Circumpedal carinae distinct, clearly not reaching the insertions of setae *3b*.

Anogenital region. Six pairs of genital (g_1, g_2, 12; g_3–g_6, 6–8), one pair of aggenital (6–8), two pairs of anal (12) and three pairs of adanal (12) setae thin, smooth. Two setae on anterior edge of each genital plate. Adanal setae ad_3 inserted postero-medially
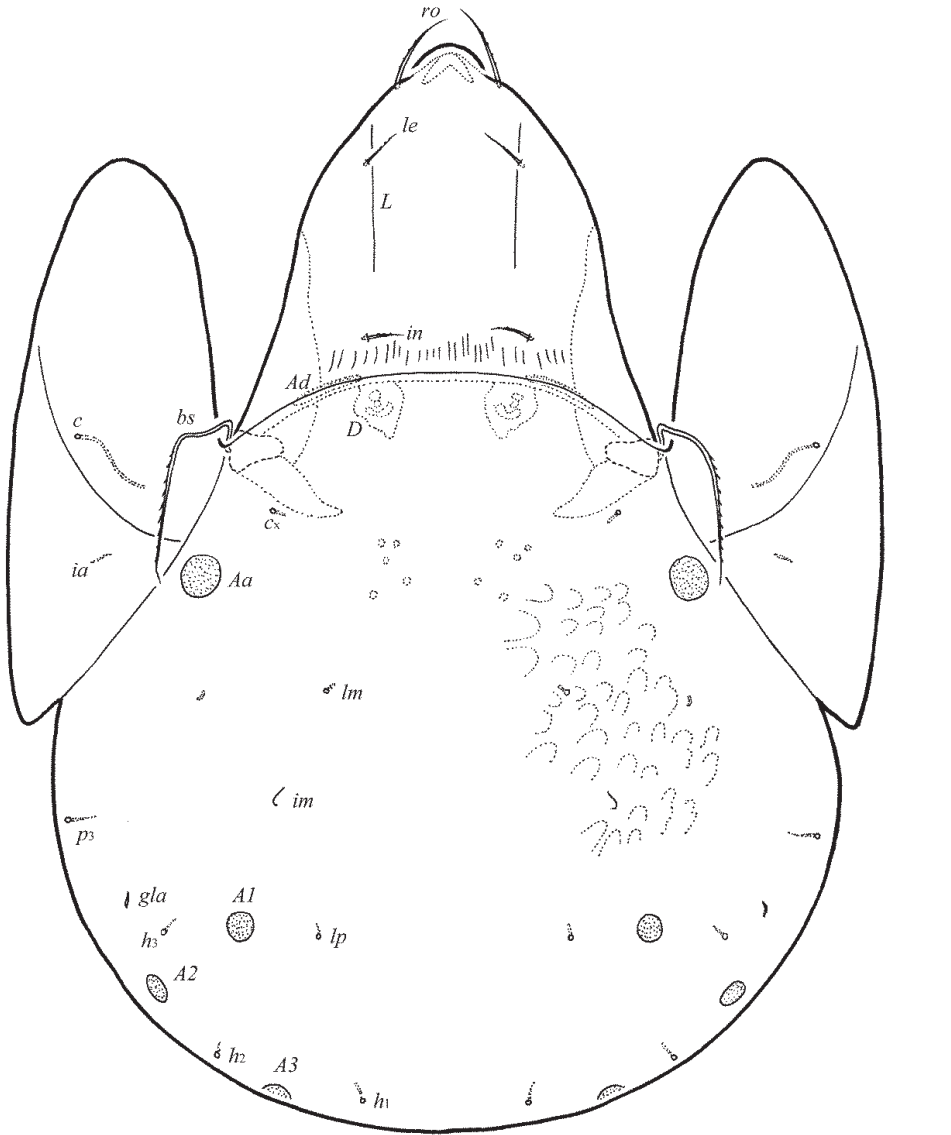
Figure 56. *Galumna* (*Neogalumna*) *specifica* sp. n., adult: dorsal view. Scale bar 100 μm.

to adanal lyrifissures. Postanal porose area elongate oval, transversally oriented (45–57 × 8–12).

Legs. Morphology of leg segments, setae and solenidia typical for *Galumna* (*Neogalumna*) (see Ermilov and Anichkin 2010, 2014b). Tridactylous, claws smooth. Formulas of leg setation and solenidia are similar to *Galumna* (*Atypicogalumna*) *corpuzrarosae* sp. n. (Table 1). Solenidion φ of tibiae IV inserted dorsally at about 2/3 length of segment.
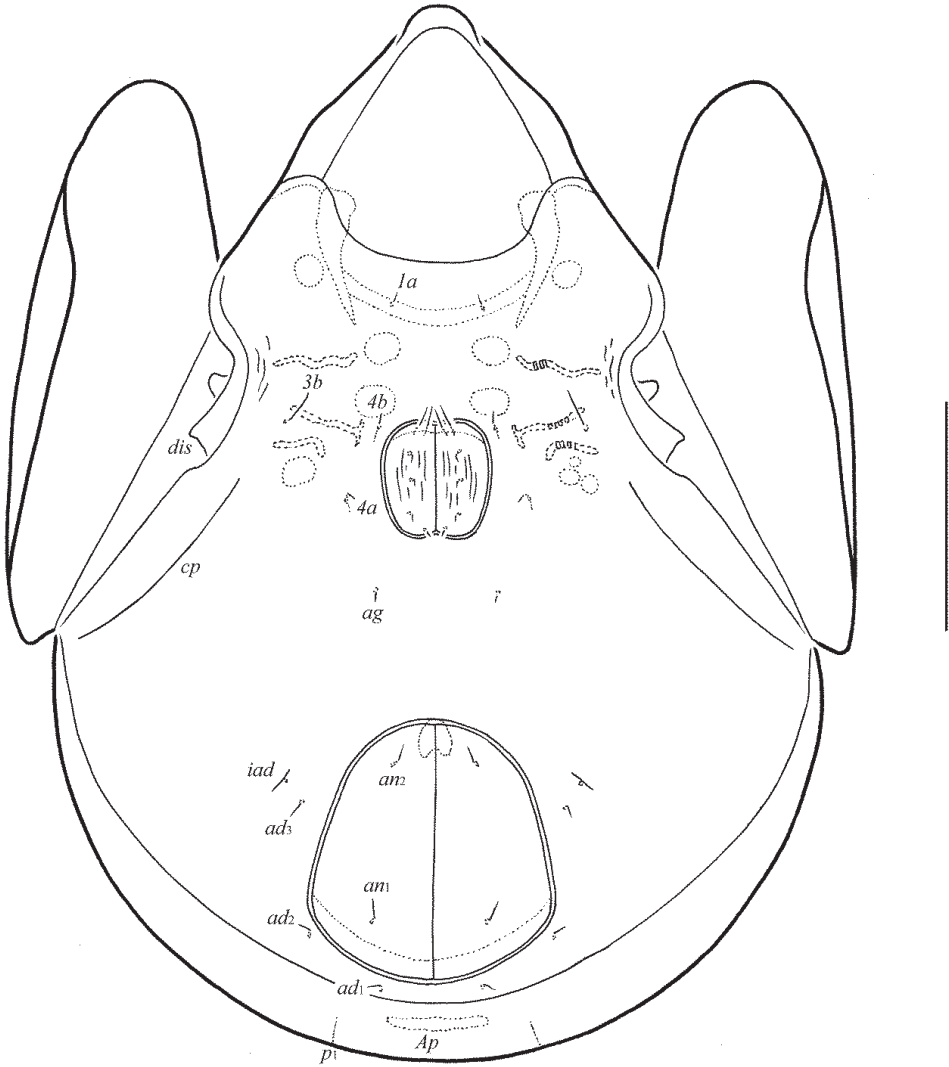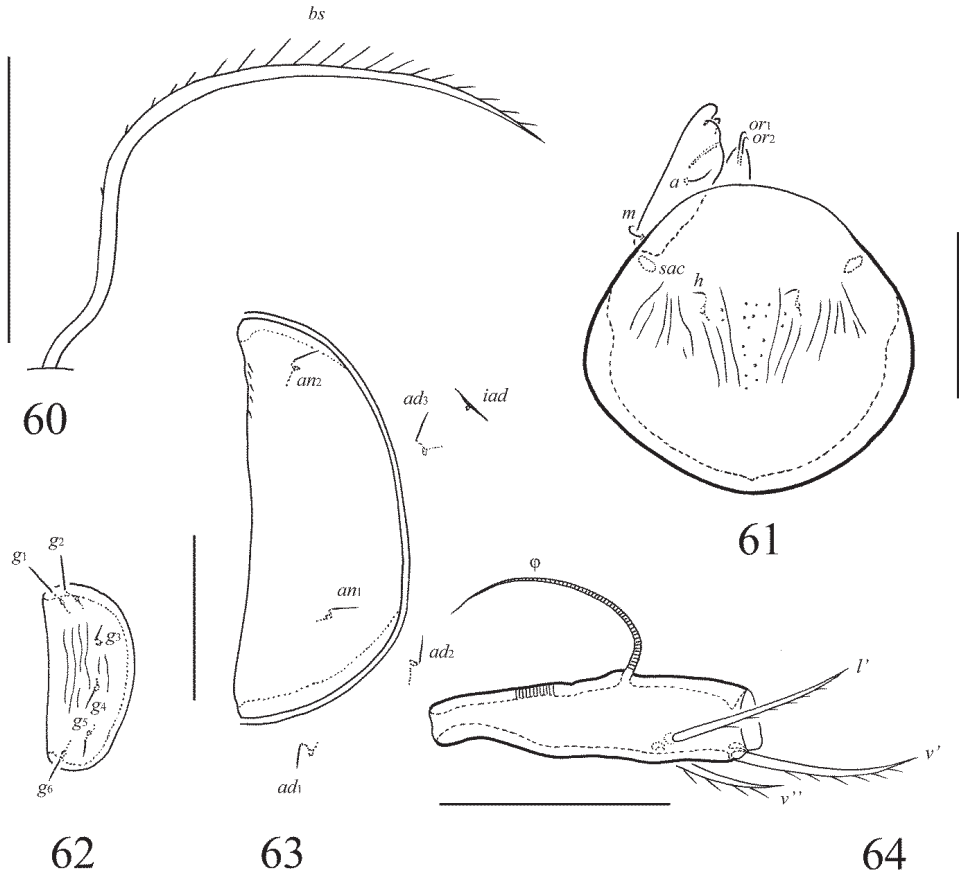
Figure 57. *Galumna* (*Neogalumna*) *specifica* sp. n., adult: ventral view (gnathosoma and legs not shown). Scale bar 100 μm.

Material examined. Holotype (male) and three paratypes (one female and three males): Indonesia, Sumatra, Harapan landscape, secondary rainforest, research site HF4, 02°11'15.2"S, 103°20'33.4"E, 77 m a.s.l., in upper soil layer (0–5 cm). All specimens were collected by Bernhard Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Type deposition. The holotype is deposited in LIPI (Indonesian Institute of Science) Cibinong, Indonesia; two paratypes are deposited in the collection of the Senckenberg Museum, Görlitz, Germany; one paratype is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.
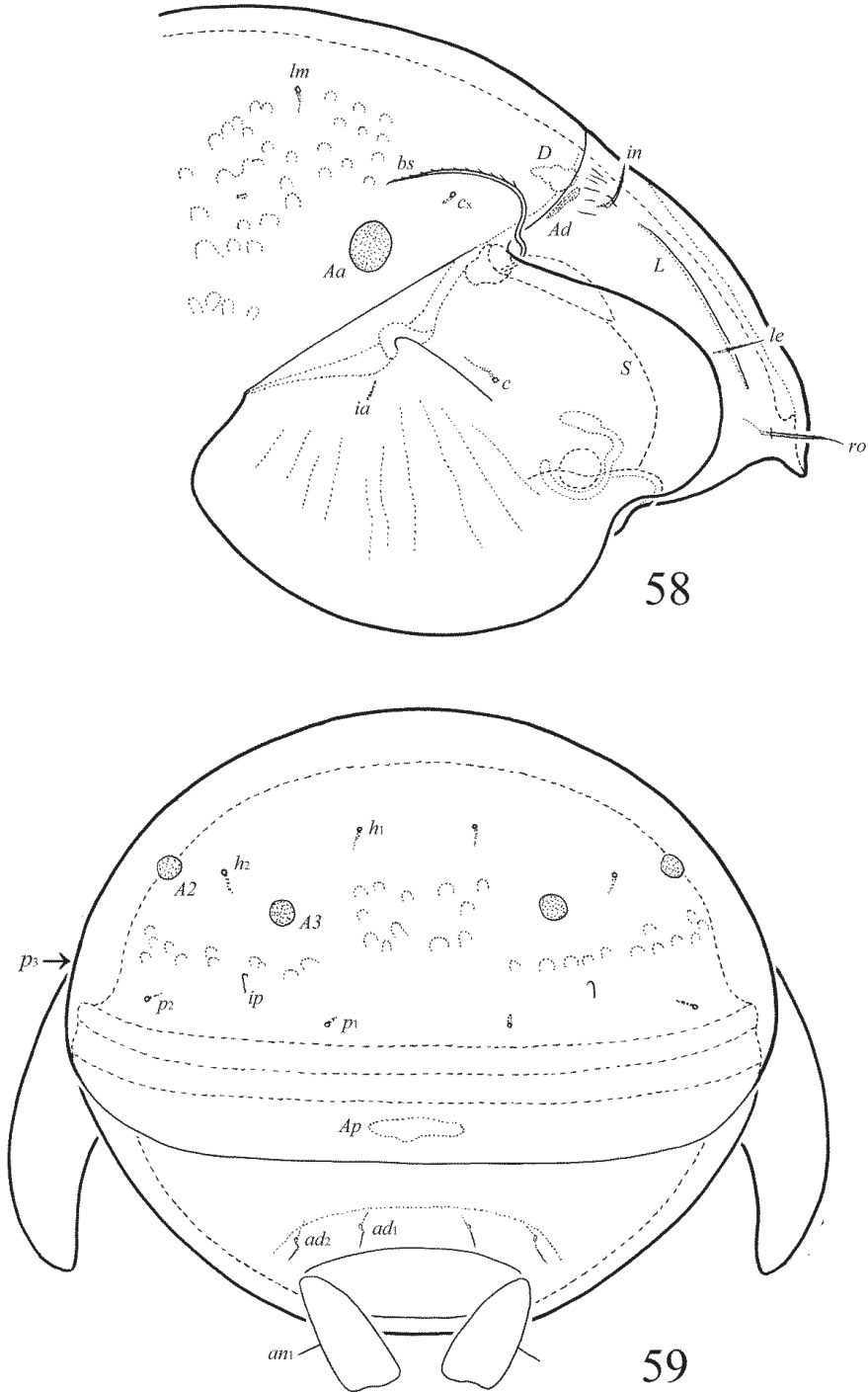
Figures 58–59. *Galumna* (*Neogalumna*) *specifica* sp. n., adult: **58** anterior part of body, lateral view (gnathosoma and leg I not shown) **59** posterior view. Scale bar 100 μm.

Figures 60–64. *Galumna* (*Neogalumna*) *specifica* sp. n., adult: **60** bothridial seta **61** subcapitulum (left gena, rutellum and lip not shown), ventral view **62** genital plate, left **63** anal plate, left, and adanal setae **64** tibia of leg IV, left, antiaxial view. Scale bars 50 μm.

Etymology. The specific name *specifica* refers to the specific set of notogastral alveoli (*la* absent, c_x present).

Remarks. *Galumna* (*Neogalumna*) *specifica* sp. n. is morphologically most similar to *G.* (*N.*) *tolstikovi* Ermilov & Anichkin, 2014 from Vietnam (see Ermilov and Anichkin 2014b) in having straight lamellar lines, short prodorsal setae, setiform bothridial setae, setal alveoli c_x and striate genital plates. However, the new species differs from the latter by larger body size (498–531 × 348–365 vs. 381–415 × 265–298 in *G.* (*N.*) *tolstikovi*), well developed and barbed interlamellar setae (vs. minute in *G.* (*N.*) *tolstikovi*), longest rostral setae on the prodorsum (vs. rostral and lamellar similar in length in *G.* (*N.*) *tolstikovi*), a striate basal part of prodorsum (vs. not striate in in *G.* (*N.*) *tolstikovi*), an elongated postanal porose area (vs. oval in *G.* (*N.*) *tolstikovi*) and the absence of setal alveoli *la* (vs. present in *G.* (*N.*) *tolstikovi*).

Records

Galumna (*Galumna*) *calva* Starý, 1996 (see Starý 1996). Distribution: Australia. New record in the Oriental region.

Material examined. Two specimens: Indonesia, Sumatra, Bukit Duabelas landscape, oil palm plantation, research site BO3, 02°04'15.2"S, 102°47'30.6"E, 71 m a.s.l., in forest floor litter material, 15.11.2013 (B. Klarner).

Galumna (*Galumna*) *flabellifera* Hammer, 1958 (see Hammer 1958; Aoki 1964, 1982; Mahunka 1978). Distribution: Pantropical and Subtropical regions.

Material examined. Two specimens: Indonesia, Sumatra, Bukit Duabelas landscape, oil palm plantation, research site BO5, 02°06'48.9"S, 102°47'44.5"E, 50 m a.s.l., in upper soil layer (0–5 cm). One specimen: same data, but in upper soil layer (0–5 cm). One specimen: Indonesia, Sumatra, Harapan landscape, rubber plantation, research site HR1, 01°54'39.5"S, 103°16'00.1"E, 77 m a.s.l., in upper soil layer (0–5 cm). All specimens were collected by B. Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

Galumna (*Galumna*) *sabahna* Mahunka, 1995 (see Mahunka 1995). Distribution: Malaysia. New record in Indonesia.

Material examined. Three specimens: Indonesia, Sumatra, Harapan landscape, oil palm plantation, research site HO1, 01°54'35.6"S, 103°15'58.3"E, 81 m a.s.l., in upper soil layer (0–5 cm). Three specimens: Indonesia, Sumatra, Bukit Duabelas landscape, research site BO3, 02°04'15.2"S, 102°47'30.6"E, 71 m a.s.l., in forest floor litter material. One specimen: Indonesia, Sumatra, Harapan landscape, rubber plantation, research site HR1, 01°54'39.5"S, 103°16'00.1"E, 77 m a.s.l., in forest floor litter material. Three specimens: Indonesia, Sumatra, Bukit Duabelas landscape, rubber plantation, research site BR2, 02°05'06.8"S, 102°47'20.7"E, 95 m a.s.l., in upper soil layer (0–5 cm). Four specimens: Indonesia, Sumatra, Bukit Duabelas landscape, rubber plantation, research site HR2, Sumatra, Indonesia, Harapan landscape, S 01°52'44.5", E 103°16'28.4", rubber plantation, 59 m a.s.l., in forest floor litter material. Three specimens: Indonesia, Sumatra, Bukit Duabelas landscape, rubber plantation, research site HR2, Sumatra, Indonesia, Harapan landscape, S 01°52'44.5", E 103°16'28.4", rubber plantation, 59 m a.s.l., in upper soil layer (0–5 cm). All specimens were collected by B. Klarner (Nov. 2013) and identified and collected to morphospecies level by Dorothee Sandmann.

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RESEARCH ARTICLE

Looking back on a decade of barcoding crustaceans

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Abstract

Species identification represents a pivotal component for large-scale biodiversity studies and conservation planning but represents a challenge for many taxa when using morphological traits only. Consequently, alternative identification methods based on molecular markers have been proposed. In this context, DNA barcoding has become a popular and accepted method for the identification of unknown animals across all life stages by comparison to a reference library. In this review we examine the progress of barcoding studies for the Crustacea using the Web of Science data base from 2003 to 2014. All references were classified in terms of taxonomy covered, subject area (identification/library, genetic variability, species descriptions, phylogenetics, methods, pseudogenes/numts), habitat, geographical area, authors, journals, citations, and the use of the Barcode of Life Data Systems (BOLD). Our analysis revealed a total number of 164 barcoding studies for crustaceans with a preference for malacostracan crustaceans, in particular Decapoda, and for building reference libraries in order to identify organisms. So far, BOLD did not establish itself as a popular informatics platform among carcinologists although it offers many advantages for standardized data storage, analyses and publication.

Keywords

Introduction

The accurate diagnosis of species represents a pivotal component for many topics, including large-scale biodiversity studies and conservation planning. Traditionally, species are identified using morphological characters. This approach requires a certain level of training in observing morphology and it usually leads to a narrow specialization in identifying organisms belonging to a restricted group of taxa (e.g. a carcinologist will likely have difficulties in identifying polychaetes and the other way around). Therefore, a routine and correct morphological identification of many taxa can be challenging, time-consuming and typically requires highly trained specialists. This is especially true for larval stages, juveniles and females which are often not included in species descriptions, resulting in a quite difficult task of assigning correct species names to specimens. In many cases morphological variability and phenotypic plasticity may also complicate a correct species determination. Furthermore, we observe a decline of taxonomists that are able to identify and characterize species of many taxa (e.g. de Carvalho et al. 2007).

As consequence of the rise of molecular biology in the last decades, the application of DNA sequence data represents a promising and effective alternative approach to identify specimens throughout all life stages (Olson et al. 1991, Caterino and Tishechkin 2006, Shank et al. 2006, Bracken-Grissom et al. 2012, Torres et al. 2014 but see Page and Hughes 2011). For animals, mitochondrial DNA (mtDNA) became highly attractive for molecular species identification due to several characteristics: generally high substitution rates, lack of introns, large copy numbers in each cell, and an almost exclusive maternal and haploid inheritance with no recombination (Ballard and Whitlock 2004, Ballard and Rand 2005, Bernt et al. 2013). In this context, a fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was proposed as so-called "DNA barcode" for animal species identification more than a decade ago (Hebert et al. 2003a). The efficacy of DNA barcoding is based on a simple assumption: each species will most likely have similar DNA barcodes representing their intraspecific variability whereas the genetic variation between species exceeds the variation within species (Hebert et al. 2003a, 2003b, 2004). In contrast to DNA taxonomy which focuses on the classification of both known and undescribed species based on sequence data only (Tautz et al. 2003, Vogler and Monagham 2007), the central aim of DNA barcoding is two-fold: 1) to assign unknown specimens to already described and classified species, and 2) to enhance the discovery of new species and facilitate identification, particularly in cryptic, microscopic, and other organisms with complex or inaccessible morphology (Hebert et al. 2003a, 2003b). Based on these assumptions, the public Barcode of Life data base (BOLD; www.boldsystems.org) acts as the core data retrieval interface, allowing researchers to collect, manage, and analyze DNA barcode data (Ratnasingham and Hebert 2007). As one of various analytical tools implemented in BOLD, barcodes can be analyzed using the Barcode Index Number (BIN) system (Ratnasingham and Hebert 2013). This approach allows a comparison of specimens identified by morphological and molecular characters.

Not surprisingly, DNA barcoding has been criticized from its beginning. In various cases, DNA barcoding was considered as a useless and expensive identification method (e.g. Will et al. 2005, Cameron et al. 2006, Ebach 2011, Taylor and Harris 2012). Other studies query methodological problems of the analysis of DNA barcodes, for example the inappropriate use of neighbor-joining trees or of fixed distance thresholds (e.g. Will and Rubinoff 2004, Goldstein and DeSalle 2010, Collins and Cruickshank 2013). Finally, another major criticism of this approach was that a single molecular marker such as COI will not necessarily provide sufficient information to deliver the resolution needed to diagnose the large number of species targeted by the initiative (e.g. DeSalle et al. 2005, Prendini 2005, Will et al. 2005). In fact, various aspects can limit the use of COI and mitochondrial DNA in general for successful species delineation. Recent speciation events, heteroplasmy, incomplete lineage sorting as consequence of phylogeographic processes, or the presence of mitochondrial pseudogenes (also known as nuclear mitochondrial DNA or numts) (e.g. Funk and Omland 2003, Bucklin et al. 2011). Furthermore, low evolutionary rates for mitochondrial genes have been demonstrated for various taxa (e.g. anthozoans and some sponges) (e.g. Shearer et al. 2002, Shearer and Coffroth 2008, Sinniger et al. 2008, McFadden et al. 2011).

Nevertheless, DNA barcoding has been successfully applied in a large number of taxonomic groups belonging to both invertebrates (e.g. Carr et al. 2011, Hausmann et al. 2011, Woodcock et al. 2013, Layton et al. 2014, Raupach et al. 2014, Raupach et al. 2015) and vertebrates (e.g. Lijtmaer et al. 2011, Ivanova et al. 2012, Knebelsberger et al. 2014). Furthermore, DNA barcodes have become an integrative part of many recently published species descriptions (e.g. Riedel et al. 2013, Khalaji-Pirbalouty and Raupach 2014, Weis et al. 2014, Hansson et al. 2015).

Within the invertebrates, the Crustacea constitute a challenging taxon for DNA barcoding. With more than 67,000 described species so far (Ahyong et al. 2013), this taxon is species-rich, morphologically diverse and ecologically important. Various crustacean species are of high economic interest (e.g. lobsters, crabs, or shrimps) and represents the basis of extensive crustacean fisheries around the world. Crustaceans can be found in all aquatic environments, and some of them successfully colonized terrestrial habitats in various degrees (e.g. talitrid amphipods, terrestrial crabs, and woodlice). However, a correct identification to the species level is not straightforward for most crustacean taxa, especially for larval and immature stages. Even as adults, numerous species are difficult to identify using morphological characters and usually require the help of taxonomists to differentiate subtle degrees of morphological variability and polymorphism within and between species. This is especially true for small deep-sea crustaceans (e.g. isopods, amphipods and tanaids), and species of the meiofauna (e.g. harpacticoid copepods).

In this review we provide an update regarding the progress of DNA barcoding in crustaceans based on descriptive statistics. Major points of the review are: taxonomic coverage, subject areas, and the use of BOLD as a major platform for the standardization of barcoding studies.

Methods

This manuscript covers research articles published between 01-01-2003 and 31-12-2014 and available in the "Web of Science" (WoS) database maintained by Thomson Reuters (http://webofknowledge.com). WoS was searched on 15-01-2015 by using "barcod*" and "crusta*" as keywords in the topic of articles hosted by all databases associated with WoS. For comparison purposes, similar searches were conducted for other arthropod taxa on the same day: Insecta ("insect*"), Chelicerata ("chelicer*") and Myriapoda ("myriapod*") in combination with "barcod*". All crustacean references were individually and carefully checked for inconsistencies, in particular false positive results (e.g. articles dealing with other taxa than crustaceans) and duplications. Only publications of the type "article" were kept for further analyses. Language was not selected as filter criterion, and non-English publications with a title and abstract in English were included. Following a strict terminology for DNA barcoding (*sensu* Hebert et al. 2003a), all articles using a different molecular marker than COI-5P' were excluded. The taxonomic focus was inferred based on the same source (titles, abstracts, keywords) and each article received a label corresponding to one crustacean order with a few exceptions: Calanoida, Harpacticoida, Cyclopoida and Siphonostomatoida were combined into "Copepoda"; Kentrogonida, Scalpelliformes and Sessilia were combined into "Cirripedia"; and the taxon Ostracoda was left at the class level. Articles that covered more than one order and did not fall into the "Copepoda" or "Cirripedia" were classified as "Crustacea". We used the recent crustacean classification of Ahyong and co-authors (2011) throughout this review as a taxonomic framework. Based on our judgment derived from reading the title, abstract, keywords and, if necessary, portions of articles, we divided all references into six subject areas: 1) identification, library (DNA barcodes used for specimen identification and/or to develop reference barcode libraries), 2) genetic variability (DNA barcodes used for studies on intraspecific genetic variability such as phylogeographic studies), 3) species description (DNA barcodes used together with morphological characters as part of species descriptions), 4) phylogenetics (DNA barcodes used in phylogenetic studies), 5) methods (new lab protocols or new primers developed for barcoding crustaceans), and 6) numts (nuclear mitochondrial DNA sequences and their implications for barcoding crustaceans). In addition and where possible, each article received a label corresponding to the habitat investigated ("marine": oceans, seas, brackish waters; "freshwater": rivers, lakes, ponds, groundwater; "mixed": marine and freshwater). Moreover, geographic labels were assigned to each article based on the main regions covered (continents and oceans). In cases of more than one ocean or continent sampled within the same article, multiple labels were assigned.

In order to verify the popularity and use of the BOLD workbench among crustacean barcoders, each article was searched for referencing BOLD and given a label: 'YES' or 'NO'. If a BOLD project was mentioned by code or title, subsequent steps were followed to find particular records in BOLD and import them into a dataset: 1) search by project code/title in BOLD Workbench, 2) copy all records from that project, and 3)

add records to dataset. All public records stored in BOLD and generated by crustacean barcoding studies can be retrieved by searching DS-CRST (Title: Crustacean Barcoding Studies) in BOLD or by going directly to the corresponding DOI associated with this dataset (http://dx.doi.org/10.5883/DS-CRST). By using a project code as search term, all records of that project were imported, regardless of its history (i.e., records added or removed from a project) between the publication date and January 2015). Some articles mentioned the use of BOLD without providing a project code. In such cases, we were able to find records by the process IDs mentioned in the publication or by searching BOLD based on taxa names. However, when tracking records was not a straightforward process, we excluded those studies from our BOLD-related analyses. DS-CRST in BOLD was used for standard barcoding analyses: number of species versus number of BINs, taxon ID tree and distance summary. Geographic coordinates, where available, were exported and used to create a map in QGIS (QGIS Development Team 2015).

Additional bibliographic data were compiled for all references: publication title, first authors' names, journal name, publication year, open-access feature, and the number of citations (as provided by WoS). The major results of our literature review are summarized graphically; a table containing all raw data is available as Suppl. material 1.

Results

Our search in WoS produced 243 hits associated with the terms "barcod*" and "crusta*", 1,064 references for "barcod*" and "insect*", 67 for "barcod*" and "chelicer*" and eight for "barcod*" and "myriapod*" (Fig. 1). In total, 1,382 publications were found for all Arthropoda. Our initial list of 243 crustacean references was revised and reduced to 164 publications after removing duplicates and mislabeled references. All other arthropod references were not revised in detail. The number of barcoding publications showed a fast increase from the first and singular crustacean article published in 2005 (Page et al. 2005) up to 30 publications in 2012 (Fig. 2). In 2013, a slight decrease to 29 publications was observed, followed by an increase to 31 publications in 2014. However, the frequencies of the different categories fluctuated each year (Fig. 2).

The taxonomic coverage of the 164 barcoding publications showed a strong preference for the Decapoda ($n = 60$, 36.7%), followed by the mixed taxon of "Crustacea" ($n = 28$, 17%), the Amphipoda ($n = 21$, 12.8%), Copepoda ($n = 18$, 11%), and Diplostraca ($n = 13$, 8%) (Table 1). All other crustacean taxa have been investigated by less than ten publications: Isopoda ($n = 6$, 3.7%), Anostraca and Cirripedia ($n = 5$, 3%), Stomatopoda ($n = 3$, 1.8%), and Bathynellacea ($n = 2$, 1.2%). The Euphausiacea, Ostracoda, and Tanaidacea have been analyzed only once (each with $n = 1$, 0.6%).

Our investigation also revealed that most crustacean barcoding studies focus on the identification of specimens and the expansion of reference libraries for various taxa ($n = 64$, 39.1%) (Table 2). Beside identification, DNA barcodes were frequently used in publications analyzing the genetic variability of species ($n = 44$, 26.8%) and as ad-
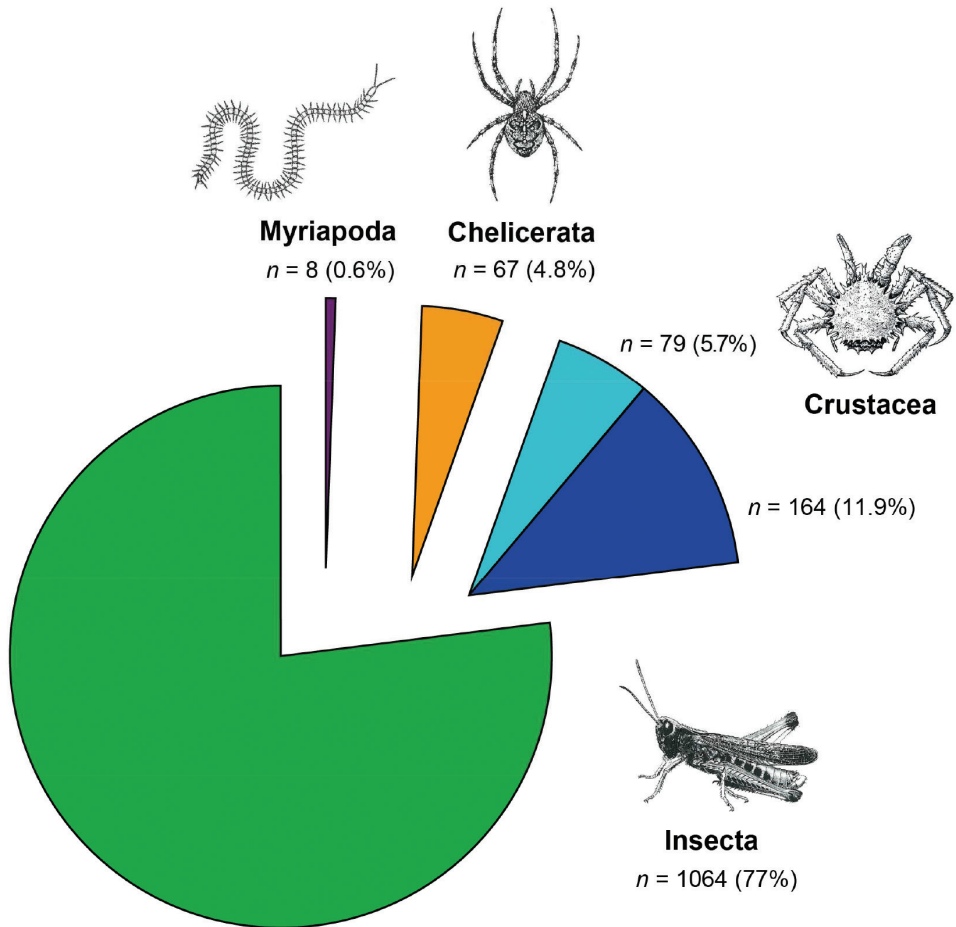
Figure 1. DNA barcoding studies of the Arthropoda. Total number and percentage values of articles published with "barcod*" and insect*" (green), "crusta*" (blue), "chelicer*" (orange), or "myriapod*" (violet) as keywords in their topic and listed in the Web of Science (period covered: 2003-2014; *n* = 1,382). For crustaceans, the total number of articles is split into: 1) the number of articles removed from our analysis (duplications and false positives) (pie sector in light blue) and 2) the core number of articles used in this review (pie sector in dark blue). Arthropod illustrations were modified from Gruner (1993) and Dathe (2003).

ditional characters in species descriptions (*n* = 32, 19.5%). Relatively small numbers of publications covered the use of DNA barcodes as part of phylogenetic reconstructions (*n* = 11, 6.7%), the publication of new protocols and methods to obtain barcode sequences (*n* = 9, 5.5%), and the study of numts (*n* = 4, 2.4%).

Approximately two thirds of the barcoding studies focused on the marine environment (*n* = 99, 60.4%) and only one third dealt with freshwater systems (*n* = 49, 29.8%) (Fig. 3). Six studies covered taxa from both marine and freshwater habitats (*n* = 6, 3.7%),
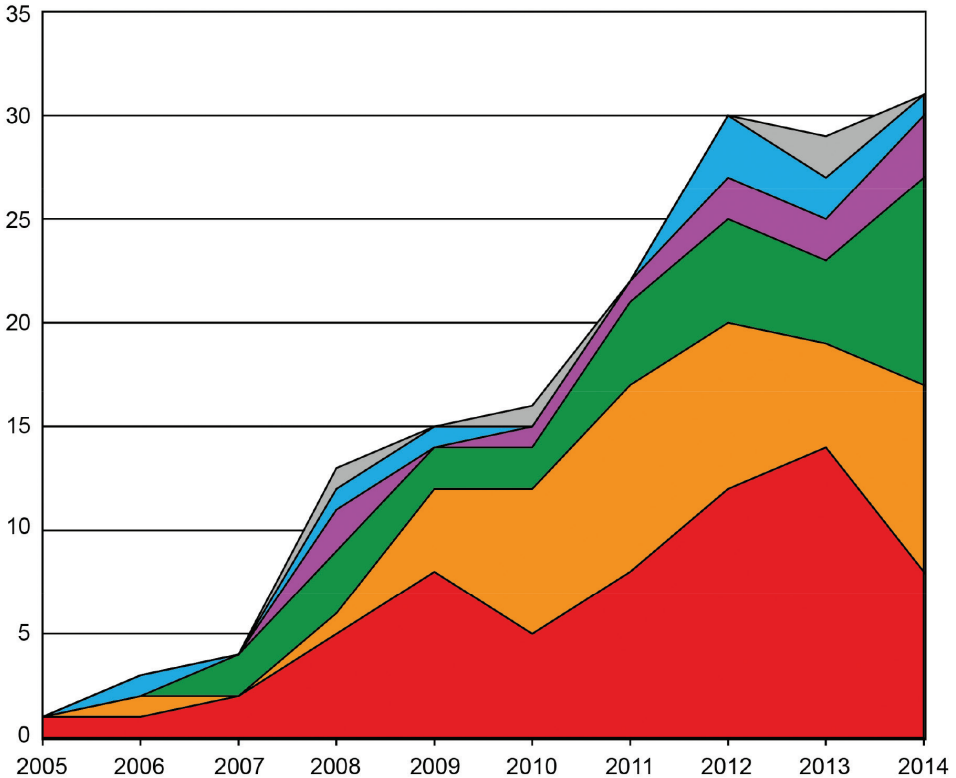
Figure 2. Subject areas of DNA barcoding studies of the Crustacea. Number of articles with "barcod*" and "crusta*" as keywords in their topic as retrieved from the Web of Science (period covered: 2003–2014; n = 164) and divided into six subject areas (from bottom to top): identification and barcode library (red), genetic variability (orange), species description (green), phylogenetics (violet), methods (blue), and numts (grey).

and for ten studies no classification was possible (6.1%). Interestingly, no study was found analyzing terrestrial crustaceans exclusively (e.g. woodlice) (Suppl. material 1). Our geographic investigation covered only the major divisions of land and water, namely continents and oceans. It should be noted that publications can include taxa from more than one environment or geographic region. The analyzed DNA barcoding publications covered all oceans (the Arctic, Atlantic, Indian, Pacific, and Southern Ocean), with a focus on the Pacific Ocean (n = 49, 25.5%), followed by the Atlantic Ocean (n = 28, 14.5%) (Fig. 3). In the case of continents, five were sampled: Asia (n = 8, 4.2%), Australia (n = 10, 5.2%), Europe (n = 17, 8.9%) as well as North and South America (n = 17, 8.9%; n = 3, 1.6%) (Fig. 3). Ten studies (5.2%) had a global geographic coverage, whereas it was impossible to place the origin of the specimens analyzed for 11 studies (5.7%), e.g. studies which used data mined from GenBank (Suppl. material 1).

The vast majority of publications (n = 129, 78.7%) did not mention BOLD in their text (label 'NO' in Suppl. material 1). The remaining 35 publications (21.3%)
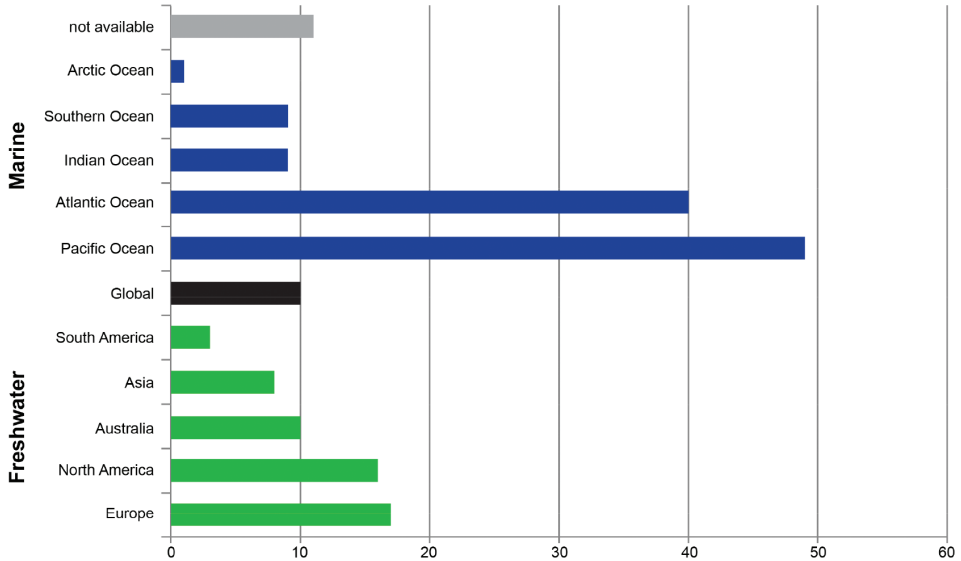
Table 1. Number of publications of the Crustacea using DNA barcodes. "Barcod*" and "crusta*" were used as keywords in the Web of Science (2003–2014). For comparison, the most recent species count per taxon is given in a separate column (based on Ahyong et al. 2011).

Taxon		Publications	(%)	Number of described species
Malacostraca	Decapoda	60	36.7	14,895
	Amphipoda	21	12.8	9,896
	Isopoda	6	3.7	10,661
	Stomatopoda	3	1.8	460
	Bathynellacea	2	1.2	241
	Euphausiacea	1	0.6	87
	Tanaidacea	1	0.6	1,069
Maxillopoda	Copepoda	18	11	15,976
	Cirripedia	5	3	1,306
Branchiopoda	Diplostraca	13	8	821
	Anostraca	5	3	313
Ostracoda		1	0.6	7,577
"Crustacea"		28	17	n. a.
Total		**164**	**100**	

Table 2. Subject area and taxonomic rank of DNA barcoding studies of the Crustacea. Number of articles were retrieved by using "barcod*" and "crusta*" as keywords in the topic of articles hosted by the Web of Science (period covered: 2003–2014).

	Identification, library	Genetic variability	Species description	Phylogenetics	Methods	numts
Decapoda	26	11	15	5	1	2
Amphipoda	4	15	1	1		
Isopoda		2	3		1	
Stomatopoda	3					
Bathynellacea		1	1			
Euphausiacea	1					
Tanaidacea	1					
Copepoda	4	5	6	3		
Cirripedia	2		2	1		
Diplostraca	2	8	3			
Anostraca	1	1	1	1	1	
Ostracoda	1					
"Crustacea"	19	1			6	2
Total	**64**	**44**	**32**	**11**	**9**	**4**

used BOLD as part of their study with project titles/codes (*n* = 23, 14%), or with projects created *a posteriori*, similar to the workflow of sequence publication in GenBank (*n* = 3, 1.8%). A handful of articles used BOLD exclusively for data mining or as an identification engine for DNA sequences or mentioned BOLD as part of current

Figure 3. Geographic and habitat focus of the analyzed DNA barcoding studies of the Crustacea. Studies were listed in the Web of Science (period covered: 2003–2014, *n* = 164), with the number of publications shown on the X axis. Green bars indicate freshwater studies, dark blue bars marine studies. A black bar represents studies that were performed on a global scale. For 11 studies, no classification was possible (grey bar). Note that publications can include taxa from more than one habitat or region.

or proposed DNA barcoding workflows. A total of 6,270 records were successfully tracked and imported into DS-CRST (Fig. 4). Approximately half of the records belonged to Malacostraca (*n* = 3,208, 51.2%), followed by Branchiopoda (*n* = 1,802, 28.8%), Maxillopoda (*n* = 728, 11.6%), and Ostracoda (*n* = 532, 8.5%). In total, 5,740 records (91.5%) had species names (Linnaean names or interim names) while 530 crustaceans (8.5%) remained unidentified (March 2015). Data owners inserted 860 species names whereas BOLD assigned 1,109 BINs to the entire dataset (Fig. 5). Furthermore, 413 records (6.6%) lacked details about the country of sample collection, 845 records (13.5%) lacked GPS coordinates whereas 3,573 specimens (57%) provided no image for the voucher. Records with collection details were divided between Canada (*n* = 2,293, 36.6%) and Mexico (*n* = 1,305, 20.8%) plus another 38 countries with much fewer records (Fig. 6). In addition to 6,270 DNA barcodes, some records used supplementary genetic markers (12S, 16S, and/or 18S rDNA). A number of 1,338 records (21.3%) had no successful chromatogram ("trace") associated, one COI sequence (0.02%) had stop codons and 45 records (0.7%) had been flagged as misidentification or contamination between the publication date and March 2015. A total of 2,082 records (33.2%) were non-barcode compliant (i.e., one of the following criteria was not fulfilled: country, two trace files, a fragment length of at least 500 base pairs, and less than 1% ambiguities).
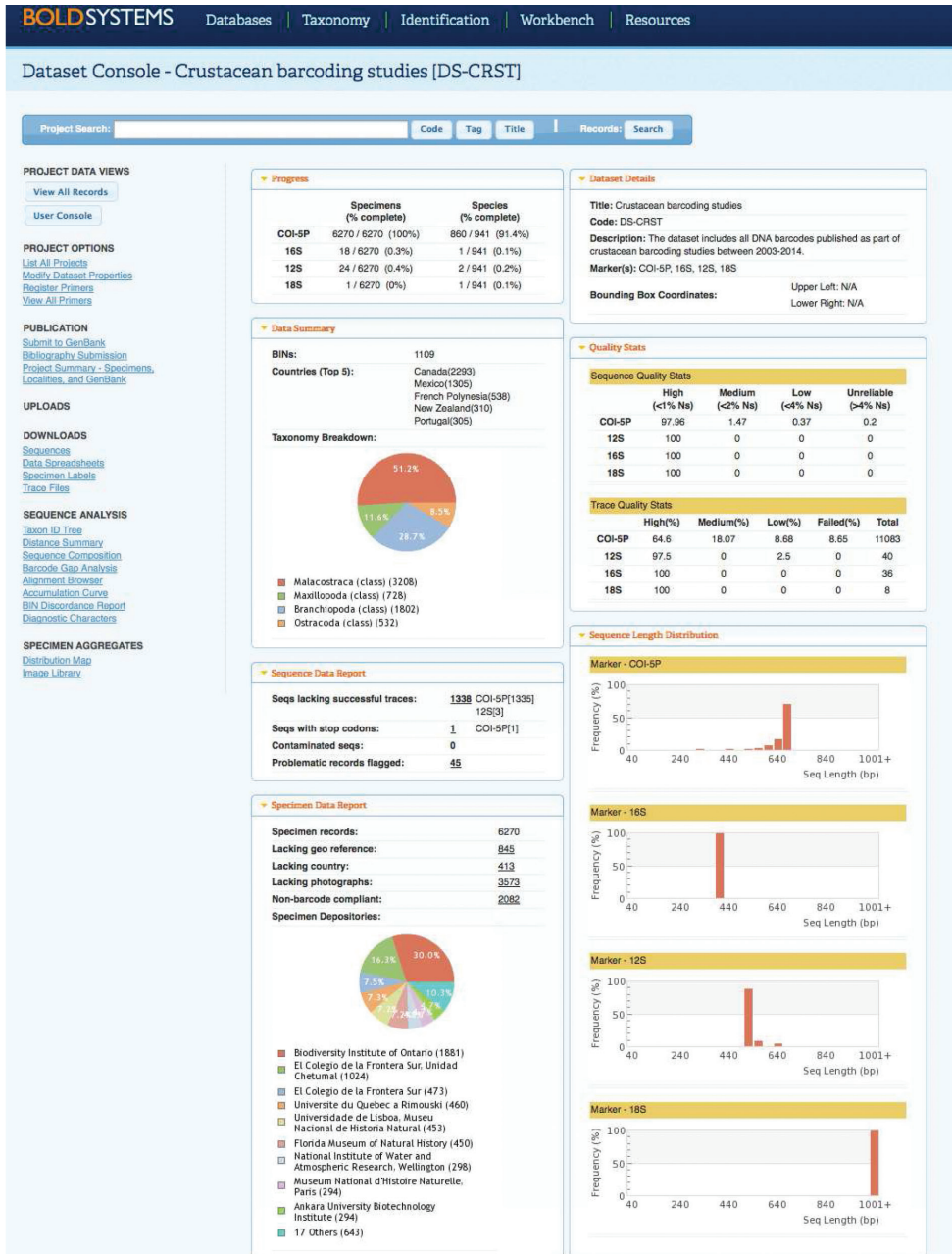
Figure 4. Project console for DS-CRST in BOLD. Various statistics for the current status of specimens are displayed: record count, species count, taxonomy breakdown, specimen depositories, country of collection, sequence count, flagged records count, trace count, image count. Note that BOLD is a dynamic environment and updates will be reflected on the project console.

Figure 5. Example for a BIN page in BOLD. The amphipod *Rhachotropis aculeata* (Lepechin, 1780) is registered in the BIN registry as BOLD:AAB3310. Note that BOLD is a dynamic environment and updates will be reflected on the BIN page, including BIN changes.

Figure 6. Sampling locations for crustaceans used in this review. GPS data was taken from the dataset DS-CRST in BOLD. Note that only 5,425 out of 6,270 records had GPS coordinates and are reflected here.

We found 76 different journals publishing articles dealing with DNA barcoding and crustaceans. Most studies were published in Zootaxa (n = 23, 14%), followed by the Journal of Crustacean Biology and PLOS ONE (each with n = 9, 5.5%), Molecular Ecology Resources (n = 7, 4.3%), Crustaceana and Invertebrate Systematics (each with n = 6, 3.7%). A number of 50 journals (65.8%) had only one article dealing with crustacean barcoding. Only 33 articles (20.1%) were open access as they were published in open access journals (e.g. PLOS ONE , ZooKeys) or in subscription journals where authors chose to publish their work as open-access (Suppl. material 1). The author list revealed a total number of 700 authors with 125 being first authors. The most prolific first author of crustaceans and DNA barcodes was Arthur Anker (7 articles in total, 4.3%), followed by Tomislav Karanovic (4 articles, 2.4%) and Ann Bucklin, Manuel Elías-Gutiérrez, Laetitia Plaisance, and Chien-Hui Yang, each with three first-authored papers involving DNA barcoding of crustaceans. The most cited article by far was written by Song and co-authors (2008) discussing the effects of numts for DNA barcoding (292 citations), followed by a publication of Lefébure and co-authors (2006) discussing threshold calculations for a successful species identification (185 citations), Witt and co-authors (2006) with one of the first articles on the role of DNA barcoding in highlighting the existence of cryptic species (172 citations), and Costa and co-authors in 2007 with the first comprehensive study testing the efficacy of DNA barcoding for crustacean species identification (165 citations) (Table 3). In the case of phylogenetic analyses using DNA barcode data the most cited article was published by Matzen da Silva and co-authors (2011a), focusing on the Malacostraca (21 citations). Finally, Lai and co-authors (2010) included DNA barcodes in their revision of the *Portunus pelagicus* (Linnaeus, 1758) species complex. This article was cited 23 times.

Table 3. Most cited crustacean barcoding articles per subject area. Data obtained from Web of Science based on a query with 'barcod*' and 'crusta*' as keywords in the topic of articles published between 2003 and 2014. Citations are given as the total number of citations since publication and the average number of citations per year (in brackets).

Subject area	Title	Authors	Journal	Year	Citations
Identification, library	Biological identifications through DNA barcodes: the case of the Crustacea	Costa FO, deWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN	Canadian Journal of Fisheries and Aquatic Sciences	2007	165 (18.3)
Genetic variability	DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation	Witt JDS, Threloff DL, Hebert PDN	Molecular Ecology	2006	172 (17.2)
Species description	A revision of the *Portunus pelagicus* (Linnaeus, 1758) species complex (Crustacea: Brachyura: Portunidae), with the recognition of four species	Lai JC, Ng PKL, Davie PJF	Raffles Bulletin of Zoology	2010	23 (3.8)
Phylogenetics	Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca)	Matzen da Silva J, Creer S, dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR	Public Library of Science ONE	2011	21 (4.2)
Methods	Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation	Lefébure T, Douady CJ, Gouy M, Gibert J	Molecular Phylogenetics and Evolution	2006	185 (18.5)
numts	Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified	Song H, Buhay JE, Whiting MF, Crandall KA	Proceedings of the National Academy of Sciences of the USA	2008	292 (36.5)

Discussion

During the past few years, crustaceans have become a popular target for DNA barcoding among the Arthropoda, being outnumbered only by barcoding studies of the Insecta (Fig. 1). Although the observed ratio of barcoding articles of insects compared to barcoding publications for crustaceans is high (6:1), this is not surprising since insects represent the most species-rich taxon on earth (app. 1 million species described and app. 5 million species estimated) (Chapman 2009). Crustacean publications showed a continuous increase starting with the first publication in 2005. In recent years, the numbers of crustacean publications seem to have reached a plateau with approximately 30 publications per year.

Although we used a highly popular database which indexes scientific literature, we are aware that an unknown number of references are missing from our study. This is mainly caused by two reasons: 1) the term "DNA barcoding" was not used in the publication although COI sequences were applied for species identification (e.g. Shih and Cai 2007), and 2) some journals might not be indexed in WoS yet. Despite this somewhat incomplete sampling of literature, we think that our review reflects the application of DNA barcodes in carcinology in a representative way.

Taxonomic overview

A rapid investigation of the taxonomic diversity covered in the 164 barcoding publications showed the highest frequency for Malacostraca (n = 94, 57.4%), the class with the largest number of crustacean species (Ahyong et al. 2011, Appeltans et al. 2012) and the most familiar ones (e.g. lobsters, crabs, shrimps, krill, beach hoppers, woodlice). Within the Malacostraca, the ecologically and economically important Decapoda were most popular for barcoding studies (n = 60 articles, 36.7%), followed by the Amphipoda (n = 21, 12.8%), a species-rich group inhabiting most aquatic habitats and even some terrestrial habitats with high humidity (e.g. supralittoral, rainforests) (Table 1). Other malacostracan orders seem to be less popular for specific DNA barcoding despite high or moderate numbers of known species, e.g. the Isopoda (10,000 species, 6 publications, 3.7%) or Tanaidacea (1,000 species, 1 publication, 0.6%). So far, no study focused specifically on the Cumacea (1,500 species) or Mysida (app. 1,200 species). We hope that scientists working on these taxa become more aware of the benefits of DNA barcoding as part of their studies, inducing an increase in the number of publications in the near future. The Maxillopoda, the second most species-rich crustacean class representing much of the marine and freshwater zooplankton, was covered in 23 studies (14%). Copepods were most popular among the maxillopods (n = 18, 11%), as it can be expected for a species-rich group (app. 16,000 species) with ecological importance in planktonic food-webs, as opposed to Cirripedia covered by only five publications (3%). The third most popular crustacean class was the Branchiopoda (n = 18, 11%), a group of crustaceans frequently encountered in freshwater habitats. Surprisingly, the species-rich class of Ostracoda (app. 7,500 species) has been covered as an exclusive taxon in only one publication (0.6%) until now. Furthermore, 28 publications (17%) had a mixture of different taxonomic groups (i.e. multiple orders were sampled) and were labeled as "Crustacea". These were usually subject-oriented (e.g. reviews on various topics) rather than taxon-oriented publications. The remaining two classes of crustaceans, Remipedia and Cephalocarida, have not been targeted by DNA barcoding studies yet (January 2015). A search using the taxon names and "COI" in GenBank returned 24 hits for the Remipedia and 20 for the Cephalocarida. Not surprisingly, these species-poor taxa (Remipedia: 18 species, Cephalocarida: 13 species; Ahyong et al. 2011) are also less important from an economic or ecological perspective. Although we do not expect comprehensive barcoding studies for species-poor taxa in the near future, we believe they might be targeted as part of comprehensive regional studies.

Subject areas of DNA barcoding publications

In contrast to the total number of publications, which revealed a steady increase followed by a relative plateau, the trend for the six subject areas (see methods) showed large fluctuations from year to year (Fig. 2). Overall, our analyses revealed that most barcoding studies focused on species identification linked to building or expanding existing reference libraries of COI sequences (n = 64, 39.1%), followed by analyses of the intraspecific genetic variability (n = 44, 26.8%) and by species descriptions that use DNA barcodes as additional characters (n = 32, 19.5%) (see Table 2). Less common were studies using DNA barcodes in molecular phylogenetics (n = 11, 6.7%), new methods and protocols (n = 9, 5.5%) or the possible effects of numts for barcoding studies of crustaceans (n = 4, 2.4%). We provide more details for each subject area in the following paragraphs.

Species identification and DNA barcode libraries

Species identification based on DNA barcodes relies on the existence of reference libraries which consist of COI sequences from specimens previously identified by experts based on traditional methods (i.e., morphological characters). Consequently, many barcoding studies published so far deal with the development of comprehensive barcode libraries (e.g. Dincă et al. 2010, Baird et al. 2011, Zhou et al. 2011, Raupach et al. 2014, Rougerie et al. 2014) and their use to identify unknown specimens (e.g. Holmes et al. 2009, Strutzenberger et al. 2011, Shen et al. 2013, Knebelsberger et al. 2014). Similar to this general trend, most crustacean publications reviewed here were found to fit in this category (n = 64, 39.1%), with a constant increase over the years (Fig. 2). In terms of crustacean diversity, most studies were performed on the Decapoda (n = 26, 40.6%) and the mixed group of "Crustacea" (n = 19, 29.9%). All the other crustacean taxa were investigated by less than five publications each (Table 2). A constantly growing library of DNA barcodes will offer numerous applications, such as seafood traceability (e.g. Haye et al. 2012, Nicolè et al. 2012, Di Pinto et al. 2013), the identification of larvae (e.g. Barber and Boyce 2006, Webb et al. 2006, Weigt et al. 2012), and tools for ecological studies in general (e.g. Valentini et al. 2009, Bowser et al. 2013, Burghart et al. 2014). Moreover, comprehensive barcode libraries will become essential for biomonitoring applications based on modern high-throughput sequencing technologies (e.g. Fonseca et al. 2010, Hajibabaei et al. 2011, Shokralla et al. 2012, Thomsen et al. 2012, Zhou et al. 2013, Leray and Knowlton 2015).

DNA barcodes and intraspecific genetic variation

The study of intraspecific genetic variation in relation to geography has become very popular in recent decades and resulted in the formation and expansion of a new research

field, namely phylogeography (Avise 2000, Hickerson et al. 2010). In the past, numerous phylogeographic studies have been published on various taxa, including crustaceans (e.g. Audzijonyte et al. 2006, Krebes et al. 2010, Campo et al. 2010, Garcia-Merchan et al. 2012, Santamaria et al. 2013). The body of sequence data generated through such phylogeographic studies was actually the background on which DNA barcoding was proposed as a method for species identification across the entire animal kingdom (Hebert et al. 2003a, 2003b). As COI sequences are used in DNA barcoding as well as in phylogeography, it is no surprise that publications with "barcod*" and "crusta*" as keywords investigate the level of genetic diversity within species as well (Fig. 2). Our review identified 44 studies for this category. Interestingly, the amphipods (n = 15, 34.1%) were more popular than decapods (n = 11, 25%) for this subject area. All other crustacean groups were present in less than ten publications per taxon (Table 2). To verify the progress in crustacean phylogeographic studies, we used phylogeograph*", "crusta*" and "cytochrome oxidase I" as keywords in WoS and retrieved 152 articles. The large discrepancy between our review and WoS is caused by the fact that the term "DNA barcode" is normally not used in phylogeographic studies as keyword. However, the variation of intraspecific genetic diversity in relation with spatial scales may have an important impact on the efficacy of DNA barcoding (Bergsten et al. 2012). Therefore we encourage researchers interested in phylogeography to address problems related to DNA barcoding as well.

New species description including DNA barcodes

Ideally, DNA barcoding and species discovery would be seen as intertwined. Whereas the main objective of DNA barcoding is to identify unknown specimens based on reference libraries, an additional outcome is reflected in the identification of unknown genetic clusters that might represent new species. As such, DNA barcodes represent powerful diagnostic supplementary characters that accelerate and revive traditional morphological taxonomy but do not replace it (DeSalle et al. 2005). It is not surprising that more and more species descriptions include barcode sequences or that entire monographs are triggered by the results of DNA barcoding (Butcher et al. 2012, Landry et al. 2013). In total, we found 32 publications incorporating DNA barcodes as part of new species descriptions of crustaceans (Table 2, Fig. 2). Again, the Decapoda were the dominant taxon (n = 15, 46.9%). Other studies focused on Copepoda (n = 6, 18.8%), Diplostraca and Isopoda (each with n = 3, 9.4%), Cirripedia (2, 6.2%), and the Amphipoda, Anostraca, and Bathynellacea (each with n = 1, 3.1%). In this context we used Thomson Reuter's Zoological Record through the Index of Organism Names (www.organismnames.com) to calculate the rate of crustacean species descriptions during the last decade. The Metrics function and the "Graphs of new taxa over time" option showed a fluctuating rate between 681 (minimum in 2014) and 1,263 (maximum in 2008) with a mean of 891 new crustacean

species being described each year, with one third representing decapods. This large discrepancy between the numbers of new species being described per year and the numbers of studies implementing DNA barcoding for species description (278:1) reflects the hesitation of taxonomists to adopt new approaches on large scale or their limited access to sequencing technologies. We hope for a change of mentality in the near future and an increased access to molecular labs as a combination of morphological and molecular data allows more detailed species descriptions as part of an aspired integrative taxonomy (e.g. Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010). In addition, the new approach would also include a standardized analytical package: raw distance data (percent divergence), diagnostic characters and phylogenetic trees (Goldstein and DeSalle 2010).

DNA barcodes and phylogenetic analyses

During the last years, COI sequences combined with other mitochondrial and nuclear markers have been frequently used to reconstruct the phylogeny of various taxa of the Crustacea (e.g. Blanco-Bercial et al. 2011, Matzen da Silva et al. 2011b, Klaus et al. 2013). Similar to phylogeographic studies, the term DNA barcode is typically not used in this context. Nevertheless, we found 11 publications using the term DNA barcodes as part of molecular phylogenetic studies, with five studies analyzing relationships of the Decapoda (45.4%), three references for the Copepoda (27.3%), and one reference for the Amphipoda, Anostraca and Cirripedia (each 9.1%), respectively (Table 2). Whereas DNA barcodes may be useful to reconstruct recent radiations and/or speciation events in some cases (e.g. Schubart et al. 1998, Cristescu and Hebert 2002), the combination of mitochondrial DNA with more conserved nuclear markers (e.g. 18S or 28S rRNA genes) is essential when reconstructing higher taxa phylogenies (Schubart 2009).

Laboratory protocols and methods

Although DNA barcoding as a molecular method for species identification has been in use for more than a decade, techniques for generating, applying, and analyzing barcode data are still being improved to guarantee an efficient workflow (e.g. Lopez and Erickson 2012). We found nine studies presenting new protocols for DNA extraction or newly designed primer pairs for crustaceans. Six publications focused on various taxa of the "Crustacea" (66.7%), and one publication for each of the remaining taxa: Anostraca, Decapoda and Isopoda (each 11.1%). As DNA barcoding becomes more and more accepted in carcinology, we are convinced that the development of more specialized protocols as well as the optimization of taxa-specific primer pairs will increase in the near future (e.g. Schubart 2009), making DNA barcoding easier and more popular for carcinologists.

Nuclear copies of mitochondrial DNA: numts

The unwanted amplification of nuclear copies of mitochondrial DNA (numts) represents a problem not only for the analyses of DNA barcodes (COI sequences) but mitochondrial genes in general (Bensasson et al. 2010, Hazakani-Covo et al. 2010). Whereas numts can be useful for phylogenetic or population structure analyses in some special cases (Pons and Vogler 2005, Hazakani-Covo 2009, Soto-Calderón et al. 2014), their presence may represent a serious problem for barcode studies. Numts are known for various taxa, including mammals (e.g. Thalmann et al. 2005, Kim et al. 2006, Soto-Calderón et al. 2014), insects (e.g. Pons and Vogler 2005, Pamilo et al. 2007, Ruiz et al. 2013, Song et al. 2014), as well as crustaceans (e.g. Schneider-Broussard and Neigel 1997, Williams and Knowlton 2001, Buhay 2009, Baeza and Fuentes 2013). Until January 2015, only four studies highlighted the potential issues of numts for DNA barcoding studies of the Crustacea, with a focus on decapods (*n* = 2, 50%) and the mixed "Crustacea" (*n* = 2, 50%). Whereas most numts were found within decapods, it is actually unclear if such pseudogenes may become problematic for other crustacean taxa too. In order to minimize the risks caused by numts for DNA barcoding studies we recommend rigorous quality control of all barcode sequences. This includes a strict use of high-quality chromatograms, a translation of the barcode sequences to amino acids to detect insertions, deletions and/or in-frame stop codons, and the use of taxa-specific primers for some groups (see Song et al. 2008, Schubart 2009).

Crustacean DNA barcoding and BOLD

In March 2015, the Public Data Portal of BOLD was hosting more than 80,000 DNA barcodes representing about 5,700 crustacean species (plus a large amount of unidentified specimens) and 10,000 BINs. Only 8% (6,270 records; 860 species names) were directly associated with crustacean barcoding studies (35 publications, Suppl. material 1) as the respective authors used BOLD for their research. The remaining crustacean barcodes were associated with private projects and with published sequences mined from GenBank. By retrieving COI data from GenBank that were generated as part of non-barcoding studies but fulfill the 'barcode' requirements, BOLD is assembling all information pertaining to reference libraries in a single database, thus reducing the risk of duplication in barcoding the same taxa multiple times. Despite a decade of work in the field of DNA barcoding, only app. 7,000 crustacean species have been barcoded to date (public and private data, available from the Taxonomy Browser in BOLD). However, existing biodiversity catalogues specify a number of more than 67,000 crustacean species described worldwide (Ahyong et al. 2011) and app. 150,000 undescribed species (Chapman 2009), although recent inventories give estimate numbers as high as 200,000–360,000 species in the marine environment alone (Appeltans et al. 2013). In times of limited taxonomic expertise as well as resources and rampant accumulation of barcode data, the option of using a DNA-based registry (such as the BIN system) for crustacean diversity has clear advantages. A

fast and accurate clustering of COI sequences into groups corresponding to presumptive species (BINs) would assist in screening large amounts of data and highlighting those cases that need detailed investigation (e.g. taxonomic synonymy, cryptic diversity, specimen misidentification). For instance, 10,000 BINs are available for crustaceans in BOLD, and a rapid initial investigation would require morphological identification of roughly 10,000 specimens as opposed to 80,000 screened through DNA sequencing. Besides identifying cohesive genetic clusters, the BIN system provides a persistent catalogue of biodiversity as each BIN has a unique alphanumeric identifier. In addition, each BIN has an individual webpage in BOLD which displays all the available information: BIN member count, nearest neighbour, genetic distance summary, haplotype network, images, sampling map, specimen depositories, collectors, identifiers, data status (public or private), data owners, annotations inserted by the barcoding community and publications using a specific BIN (Fig. 5). Multiple options to download specimen and/or sequence data are also given.

A growing database such as BOLD, which follows specific high standards for data quality, will certainly be useful for large-scale analyses in crustacean phylogeography, biogeography and biodiversity assessment and will offer support for technological advances such as high-throughput sequencing.

Conclusions

Our review shows that DNA barcoding has gained popularity in carcinology and that the most popular group targeted for various related topics are the malacostracan crustaceans, in particular decapods. As the main goal of DNA barcoding is to assign unknown specimens to known species, most crustacean barcoding studies were found to build or use existing reference libraries for identification purposes and this trend will surely continue and probably increase in the future. The generation of comprehensive barcode libraries will represent a challenging but also an important task, especially for some species-rich habitats (e.g. the deep sea or coral reefs), where our general knowledge about crustacean diversity, in particular species numbers, is still poor. A second objective of DNA barcoding is to accelerate species discovery, particularly in cryptic, microscopic and other organisms with complex or inaccessible morphology. We believe that more progress will be made in this direction as well.

Crustacean taxonomy seems to be slowly incorporating DNA barcoding in the field as the top journal in this field is a taxonomic journal and the most prolific first authors have a taxonomic background. However, a larger acceptance and application is highly desirable, and therefore we encourage a stronger cooperation between "classical" taxonomists and the DNA barcoding community. Moreover, the term "DNA barcode" should only be used for COI-5P` sequences (Hebert et al. 2003a). In this context we also recommend the use of BOLD for data storage, analysis and publication. By following such standards in data generation and analysis, large comparisons across taxonomic groups would be easily drawn for better predictions of biodiversity, in particular molecular, patterns and species diversity in general.

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Supplementary material 1

A decade of DNA barcoding of crustaceans: input file
Authors: Michael J. Raupach, Adriana Radulovici
Data type: data table
Explanation note: Raw data related to 164 publications on crustacean barcoding as retrieved from Web of Science: bibliography, citations, habitat type, geographical area, BOLD use.

RESEARCH ARTICLE

Leucothoe kawesqari, a new amphipod from Bernardo O'Higgins National Park (Chile), with remarks on the genus in the Magellan Region (Crustacea, Peracarida)

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Abstract

Although the genus *Leucothoe* has been reported repeatedly in the Magellan Region, the citations in the Channels and Fjords Ecoregion were either unidentified or attributed to the previously considered cosmopolitan *Leucothoe spinicarpa*. In this work, *Leucothoe kawesqari* **sp. n.** is described, which can be distinguished from other species of the genus in the Southern Ocean by having eyes present, epimeral plates with no setae, anterior coxae not acutely produced or excavate, coxa 5 slightly bilobed, accessory flagellum present, mandibular palp article 3 shorter than ½ article 2, pereopods 5–7 basis expanded, ovoid, posterior margin weakly crenulate and telson apex irregularly truncated. The new species was found in hard substrates, both unvegetated and with macroalgae, mainly in kelp forest of *Macrocystis pyrifera*.

Resumen

A pesar de que el género *Leucothoe* ha sido citado en la región Magallánica en repetidas ocasiones, las citas en la Ecorregión de Canales y Fiordos o bien no han sido identificados o atribuidos a la antes considerada especie cosmopolita *L. spinicarpa*. En este trabajo se describe *L. kawesqari* **sp. n.**, que se distingue de otras especies del género que se encuentran en el Océano Antártico por presentar ojos, placas epimerales sin setas, coxas anteriores no puntiagudas ni excavadas, coxa 5 ligeramente bilobulada, flagelo accesorio, artículo 3 del palpo mandibular más corto que la mitad del artículo 2, bases de los pereiópodos 2–7 expandidas con el margen ligeramente crenulado y ápice del telson truncado e irregular. La nueva especie fue encontrada en sustratos duros, tanto sin vegetación como con macroalgas, dominadas por bosques de huiros de la especie *Macrocystis pyrifera*.

Introduction

Leucothoe Leach, 1814 is a speciose amphipod genus that currently comprises 132 species (WoRMS 2015). Species of *Leucothoe* are widespread in all oceans and inhabit a variety of benthic environments from the intertidal zone to –3570 m although most of them live in shallow waters in association with other invertebrates such as in ascidians, sponges, and bivalve mollusks, or free living in algae or coral rubble (White 2011).

The genus *Leucothoe* has been reported repeatedly in the Magellan Region, defined as the Patagonian shelf south of about 41°S on both the Pacific and Atlantic sides, assigned to the wrongly considered cosmopolitan species *Leucothoe spinicarpa* (Abildgaard, 1789) (see De Broyer et al. 2007, White 2011). Krapp-Schickel and De Broyer (2014) revised the *Leucothoe* in the Southern Ocean, and clarified the citation of Holman and Watling (1983) from Tierra de Fuego by describing the species *Leucothoe weddellensis* Krapp-Schickel & De Broyer, 2014. The rest of the records of the genus in the region do not provide descriptions, hence the specimens cannot be attributed to any described species. In this work, a new species found in the channels and fjords of Bernardo O'Higgins National Park (henceforward BONP) is described.

Materials and methods

BONP is placed in the Chilean geopolitical regions of Aysén and Magallanes, between 48.0–51.6°S and 73.3–75.8°W (Aravena et al. 2011). Its coastal line consists of countless channels and fjords along more than 400 linear kilometers of the southeastern Pacific (Aldea et al. 2011), which house a variety of habitats.

Between January and March 2010 two cruises were carried out onboard the vessel *MV Nueva Galicia* with the objective of sampling the rocky sublittoral bottoms of the channels and fjords of BONP. A total of 23 sites was sampled with SCUBA: five samples were taken manually at both 5 and 15 m depth at each site (10 samples at each site), harvesting squares of 25×25 cm (0.063 m^2) by scraping off all the organisms (including fauna and smaller algae), but not the kelps. Samples were fixed in 5% buffered formalin and subsequently sorted, preserved in 70% alcohol and identified. Illustrations were performed using a *camera lucida* connected to a compound microscope.

Terminology used in the description follows Krapp-Schickel and De Broyer (2014). Body length is measured from dorsodistal extreme of pereon to frontal tip of head. Specimens with no penile process or marsupium are considered neuters. Type

material is lodged in the collections of the Museo Nacional de Historia Natural, Chile (MNHNCL) and the Museo Nacional de Ciencias Naturales de Madrid (MNCN), Spain.

Results

Systematics

Order Amphipoda Latreille, 1816
Suborder Gammaridea Latreille, 1802
Family Leucothoidae Dana, 1852
Genus *Leucothoe* Leach, 1814

***Leucothoe kawesqari* sp. n.**
http://zoobank.org/B87DCE33-A922-4252-96AD-478362B416B1

Material examined. Holotype: female, 8 mm length, MNHNCL AMP-15038; Paratypes: female, dissected, 5 mm length, MNHNCL AMP-15039; 2 females, MNCN 20.04/10146, MNCN 20.04/10147; All, 51°04'04.7"S, 74°08'29.5"W, 5–15 m depth, rocks, 27 January 2010. Other material examined: 8 females and neuters, 51°04'04.7"S, 74°08'29.5"W, 5–15 m depth, rocks, 27 January 2010; 16 neuters, 49°36'16.7"S, 75°23'31.4"W, 5–15 m depth, rocks with macroalgae, 19 March 2010, 1 female, 49°11'27.1"S, 75°23'30.8"W, 5–15 m depth, rocks with macroalgae, 19 March 2010. All coll. R. Barría, E. Newcombe, M. Hüne and T. Césped.

Diagnosis. Head anterior margin rounded, mid-cephalic keel quadrate but not prominent. Eyes present. Epimeral plates with no setae, epimeron 3 posterior margin excavate, postero-ventral corner blunt, with right angle. Coxae 1-3 not acutely produced, nor excavated, coxa 3 longer than broad, coxa 5 slightly bilobed. Antenna 1 main flagellum of 11–12 articles, accessory flagellum present, minute, of two unequal segments. Antenna 2 flagellum of 6 articles. Mandibular palp article 3 shorter than ½ of article 2. Ganthopod 1 propodus palm with minute serrations. Dactylus reaching 0.4 of propodus length. Gnathopod 2 basis posterior margin smooth, carpus smooth, without tooth or process, setose, reaching 0.3 of propodus length, propodus with short, blunt distal prolongation and three medial rows of setae. Pereopods 5–7 bases expanded, ovoid, posterior margin weakly crenulate. Telson apex irregularly truncated.

Derivatio nominis. Named after the Alacalufe people Kawésqar, whose ancestral territory extends through the Magellan Region, from the Gulf of Penas to the Strait of Magellan.

Description.

Body (Fig. 1A) length 5–8 mm.

Head (Fig. 1A) anterior margin rounded, anterodistal margin rounded. Mid cephalic keel quadrate but not prominent, eyes present, rounded.
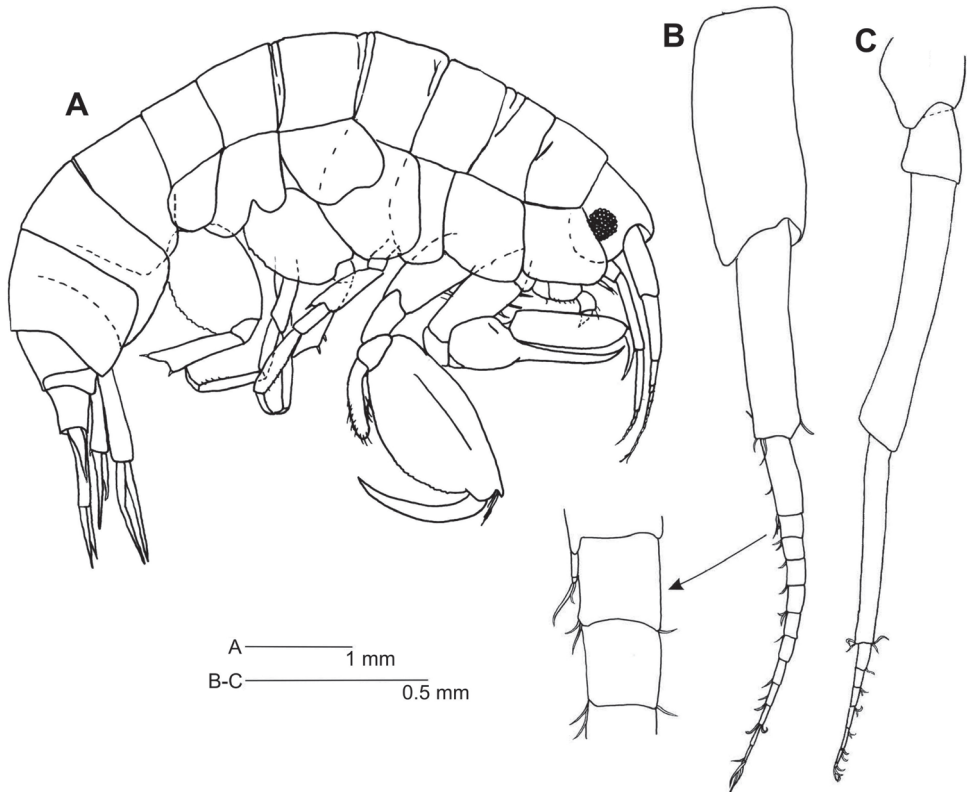
Figure 1. *Leucothoe kawesqari* sp. n. **A** Habitus **B** Antenna 1 **C** Antenna 2.

Antenna 1 (Fig. 1B) 0.4 times as long as body; peduncle article 1 2.3 times as long as broad; article 2 subequal in length, 4.3 times as long as broad; article 3 half as long as long article 2, 2.8 times as long as broad; accessory flagellum present, minute, about ½ as long as main flagellum article 1, biarticulated, first article about three times as long as second; main flagellum of 11 articles, about as long as peduncle article 1, aesthetascs present, flagellum article 1 as long as articles 2–3 and half of 4 together.

Antenna 2 (Fig. 1B) slightly shorter than antenna 1; peduncular article 5 0.8 times as long as article 4; flagellum of 6 articles.

Mouthparts. Upper lip (Fig. 2A) asymmetrically lobate, anterior margin setose. Mandibles (Fig. 2B, C) lacking molars; mandibular palp (Fig. 2D) article 2 with 9 lateral and 3 distal setae, article 3 about 1/3 of article 2, with two unequal distal setae; incisor dentate, spine row of 12 serrate spines; left lacinia mobilis (Fig. 2B) large, distally as long as incisor; right lacinia mobilis (Fig. 2C) small, weakly dentate, Maxilla 1 (Fig. 2E) palp two-articulate, distal article with three distal spines; outer plate with four distal spines, three distal slender spines and three subdistal fine setae; inner plate with one distal small seta. Maxilla 2 (Fig. 2F) outer plate with three distal spines, outer margin subdistally setulose; inner plate with nine spines along inner margin. Maxil-
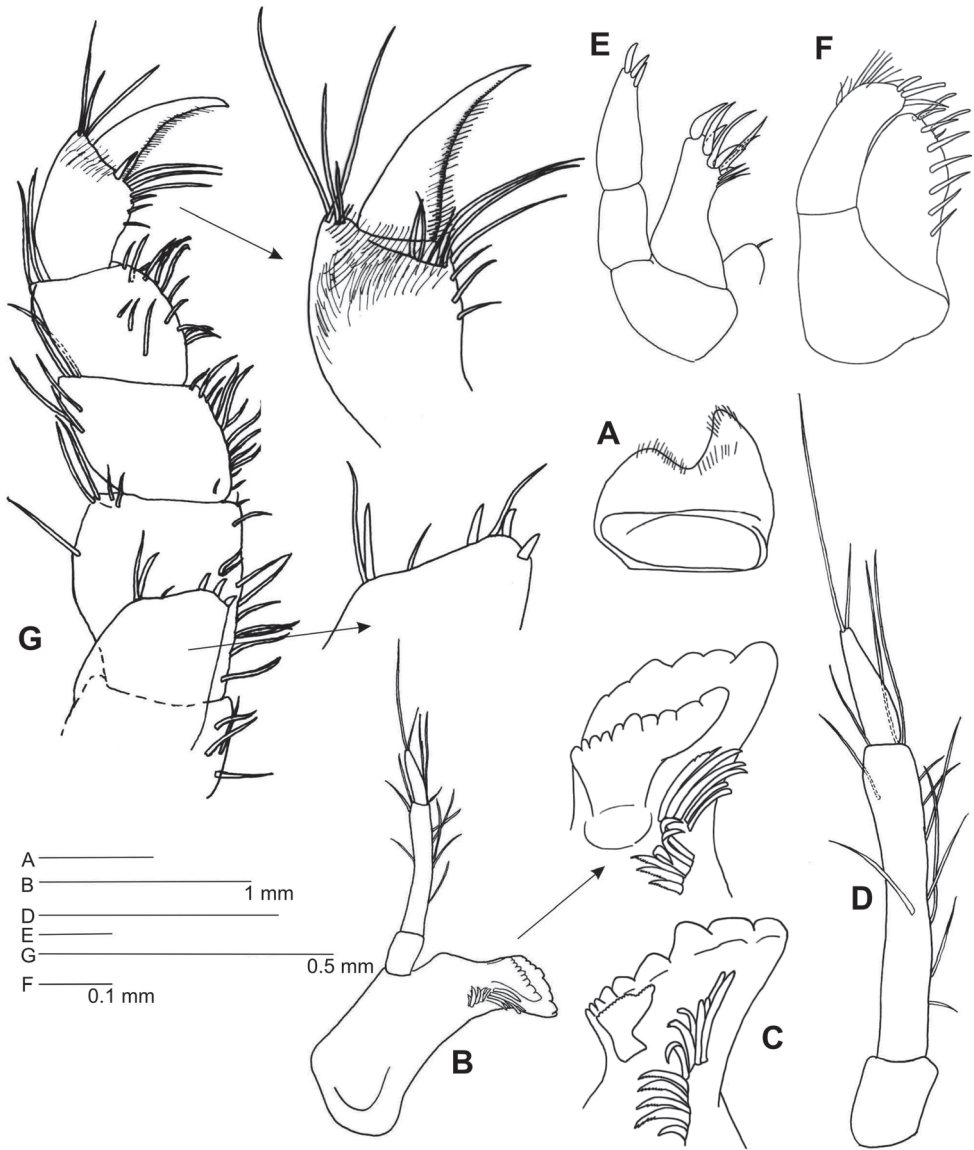
Figure 2. *Leucothoe kawesqari* sp. n. **A** Upper lip **B** Left mandible **C** Right mandible **D** Mandibular palp **E** Maxilla 1 **F** Maxilla 2 **G** Maxilliped.

liped (Fig. 2G) inner plate distal margin with three setae, three short spines on inner corner and one slender spine on outer corner; palp articles 2–4 similar in length.

Gnathopod 1 (Fig. 3A) coxa naked, anterior margin 1.4 as long as posterior; basis anterior and posterior margins with sparse short setae; ischium naked; carpus linear, naked; propodus 3.6 times as long as broad, palm with minute serrations and row of 7 short setae; dactylus smooth, reaching 0.4 length of propodus.

Gnathopod 2 (Fig. 3B) coxa subquadrate, naked; basis anterior margin with 4–5 setae, posterodistal corner with one seta; carpus reaching 0.3 of propodus length, curved, distally rounded, densely setose; propodus twice as long as broad, anterodistal margin with short, blunt prolongation bearing a tuft of setae, with three facial rows of setae, osp. n.rse and near palm, one mediofacial and reaching 2/3 the length of propodus, and one displaced dorsally, reaching from distal corner to 1/3 length of propodus, palm convex, slightly crenulated; dactylus smooth, reaching 0.6 length of propodus.

Pereopod 3 (Fig. 4A) coxa longer than broad, distal margin rounded, naked; basis very narrow, naked; merus with anterodistal spine; propodus with a row of six ventral, short spines.

Pereopod 4 (Fig. 4B) coxa naked, anterior margin longer than posterior, distal margin rounded and oblique, posterior margin tapered; propodus with a row of nine ventral, short spines; otherwise as pereopod 3.

Pereopod 5 (Fig. 4C) coxa naked, slightly bilobed; basis oval, 1.5 times as long as broad, anterior margin with spines, posterior margin weakly crenulated; merus anterior and posterior margins with spines, posterodistal corner lengthened; propodus anterior margin with a row of spinules.

Pereopod 6 (Fig. 4D) coxa bilobed; basis 1.4 times as long as broad; otherwise as pereopod 5.

Pereopod 7 (Fig. 4E) coxa shorter than broad, distal margin rounded; otherwise as pereopod 6.

Epimeral plates (Fig. 1A) naked; epimeron 2 posterior margin concave, posteroventral corner without cusp; epimeron 3 posterior margin excavate, postero-ventral corner blunt, with right angle.

Uropods. Uropod 1 (Fig. 4F) peduncle 0.7 times as long as outer ramus; outer ramus with 5 spines; rami with marginal spines, inner ramus slightly shorter than outer. Uropod 2 (Fig. 4G) somewhat shorter than uropod 1, peduncle 1.6 times as long as outer ramus, with spines on distal half of outer margin; rami with marginal spines, inner ramus slightly shorter. Uropod 3 (Fig. 4H) 1.1 times as long as uropod 1, peduncle 1.2 times as long as outer ramus; outer ramus with marginal spines; the shorter one 0.8 times as long as the longer one, naked.

Telson (Fig. 4I) 3.3 times as long as broad, distal tip minutely, irregularly truncate.

Remarks. As mentioned above, the only described species of *Leucothoe* found in the Magellan Region is *L. weddellensis*. Following Holman and Watling (1983), Krapp-Schickel and De Broyer (2014) divided the material of *L. weddellensis* in two groups, according to morphological differences, locations and size. The material from the Magellan Region (South of Tierra de Fuego) corresponds to the larger specimens (more than 14 mm long). From those, *L. kawesqari* differs (besides the length) in that the former lacks accessory flagellum, has a distinctively more slender gnathopod 1 propodus, coxa 5 is markedly bilobed, peropods 5–7 basis are pear-shaped oval (while in *L. kawesqari* are regularly oval) and more slender, the epimeron 1 is posteriorly serrate, the epimeron 2 has ventrodistal setae, and epimeron 3 as a posterodistal small prolongation. The smaller specimens differ in lacking accessory flagellum, having a

longer mandibular palp article 3 (1/2 of the length of article 2), gnathopod 1 propodus anterior margin concave, gnathopod 2 basis more setose, pereopods 5–7 distinctly narrower, pereopods 5–6 with slightly concave hind margin.

L. kawesqari is most similar to *Leucothoe antarctica* Pfeffer, 1888 as redescribed by Krapp-Schickel and De Broyer (2014): they share a mandibular palp article 3 1/3 length of article 2, coxa 3 longer than broad with rounded distal margin, and 5–7 basis oval, but *L. antarctica* lacks accessory flagellum, has a coxa 5 distinctively bilobed, maxilliped palp article 4 and 5 more slender, setae on gnathopod 2 ischium and merus, pereopod 6 basis strongly serrated, epimeron 2–3 with ventrodistal setae, uropods more spinose and telson with a pair of distal setae.

Regarding other species from the Southern Ocean, *Leucothoe merletta* Krapp-Schickel & De Broyer, 2014 can be readily differentiated from *L. kawesqari* because of having coxae 2 and 4 with acute anterodistal angles, having mandibular palp article 3 about as long as article 2, pereopods 5–7 basis with regularly rounded hind margin, epimeron 1 distal margin rounded and epimeron 3 with rectangular posterodistal corner. *Leucothoe campbelli* Krapp-Schickel & De Broyer, 2014 has a longer mandibular palp article 3 (1/2 length of article 2), coxa 3 subtrapezoidal, gnathopod 1 propodus more robust, pereopods 5–7 basis posterior margin smoth and epimeral plate with posterodistally upturned corner. *Leucothoe longimembris* Krapp-Schickel & De Broyer, 2014 lacks eyes, no accessory flagellum, mandibular palp article 3 1/2 length of article 2, and basis of pereopods 5–7 slim, broadest proximally. *Leucothoe macquariae* Krapp-Schickel & De Broyer, 2014 lacks accessory flagellum, mandibular palp article 3 1/2 length of article 2, more robust gnathopods carpi, epimeron 3 distal posterior margin distal corner blunt and upturned, and telson tip acute. *Leucothoe orkneyi* Holman & Watling, 1983 can be immediately differentiated in having a very slender gnathopod 1 propodus, and having a very prominent mid-cephalic keel, no accessory flagellum, a very slender, pereopods 5–7 basis margin strongly serrated, and epimera distal margins with setae.

Leucothoe tolkieni Vinogradov, 1990 is the only other species described from the Southeastern Pacific, although it was found well offshore. It differs from the species described here mainly in having the head anterior margin truncate with eyes that cover most of the head, gnathopod 1 basis anteroproximally expanded, and propodus curved, proximally inflated, gnathopod 2 carpus distally truncate, spoon-like, pereopods 5–7 bases narrowly expanded and telson apex rounded.

Although previous reports of *L. spinicarpa* in the Magellan Region are probably wrong (De Broyer et al. 2007, White 2011), it is worth mentioning the main differences with the present species. Based on the description provided by Crowe (2006), unlike *L. kawesqari*, *L. spinicarpa* has a gnathopod 1 propodus ventral margin with more than 10 spines, coxa 5 markedly bilobed, gnathopod 2 carpus scarcely setose with a subdistal cusp, epimeron 1 with anterodistal tuft of setae, and telson apex bidentate with a pair of distal setae.

Ecology. *Leucothoe kawesqari* was one of the dominant species of amphipod found in unvegetated hard substrates in the southernmost sampling site, where the
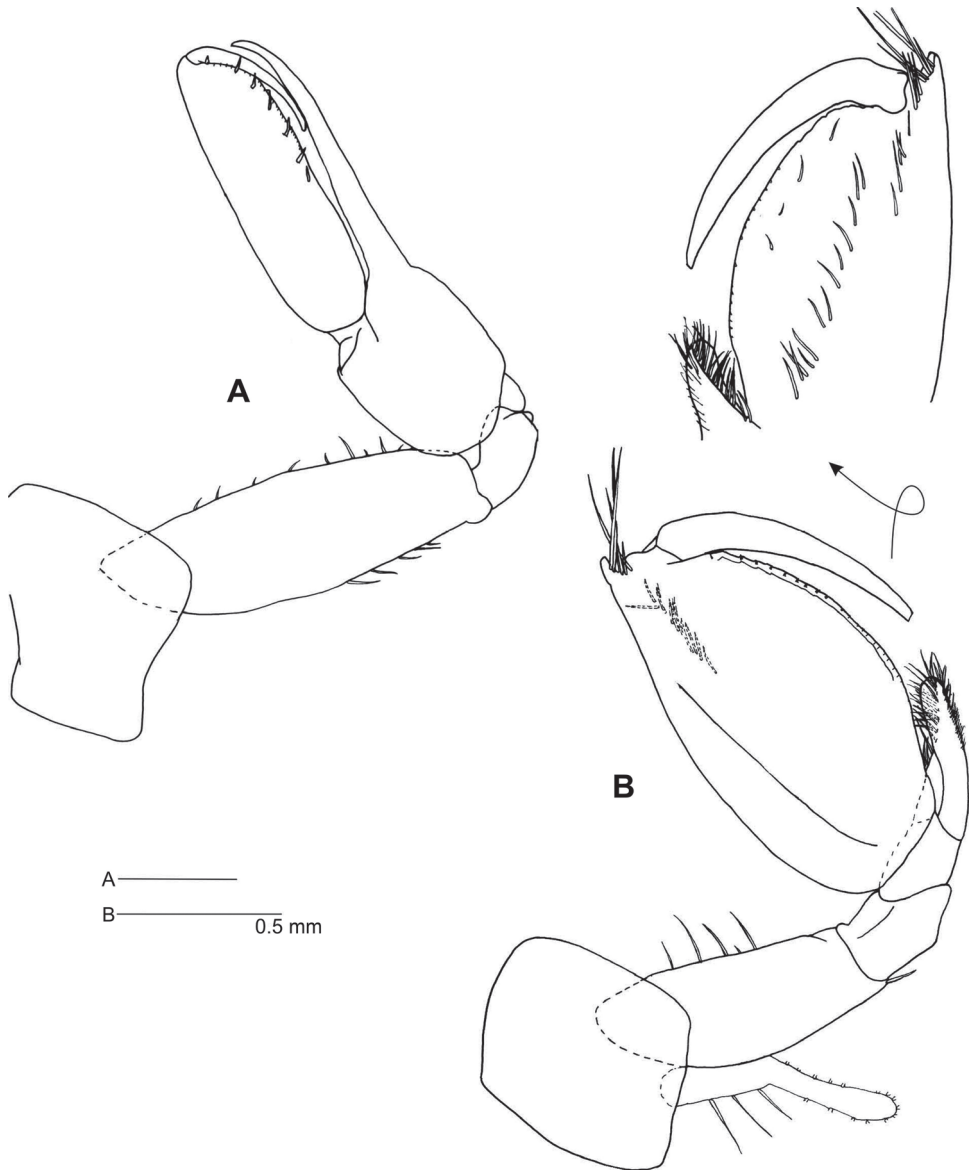
Figure 3. *Leucothoe kawesqari* sp. n. **A** Ganthopod 1 **B** Gnathopod 2.

amphipods *Polycheria antarctica* (Stebbing, 1875) and *Orchestia* spp., were also abundant. Towards the north of BONP, *L. kawesqari* was found in subtrates dominated by kelp forest of *Macrocystis pyrifera,* where *Andaniopsis integripes* (Bellan-Santini & Ledoyer, 1986) was dominant and it also co-occurred with the tanaid *Zeuxoides troncosoi* Esquete & Bamber, 2012 and juveniles of the isopod family Janiridae. High abundances of other benthic taxa were found co-occurring with *L. kawesqari*: the
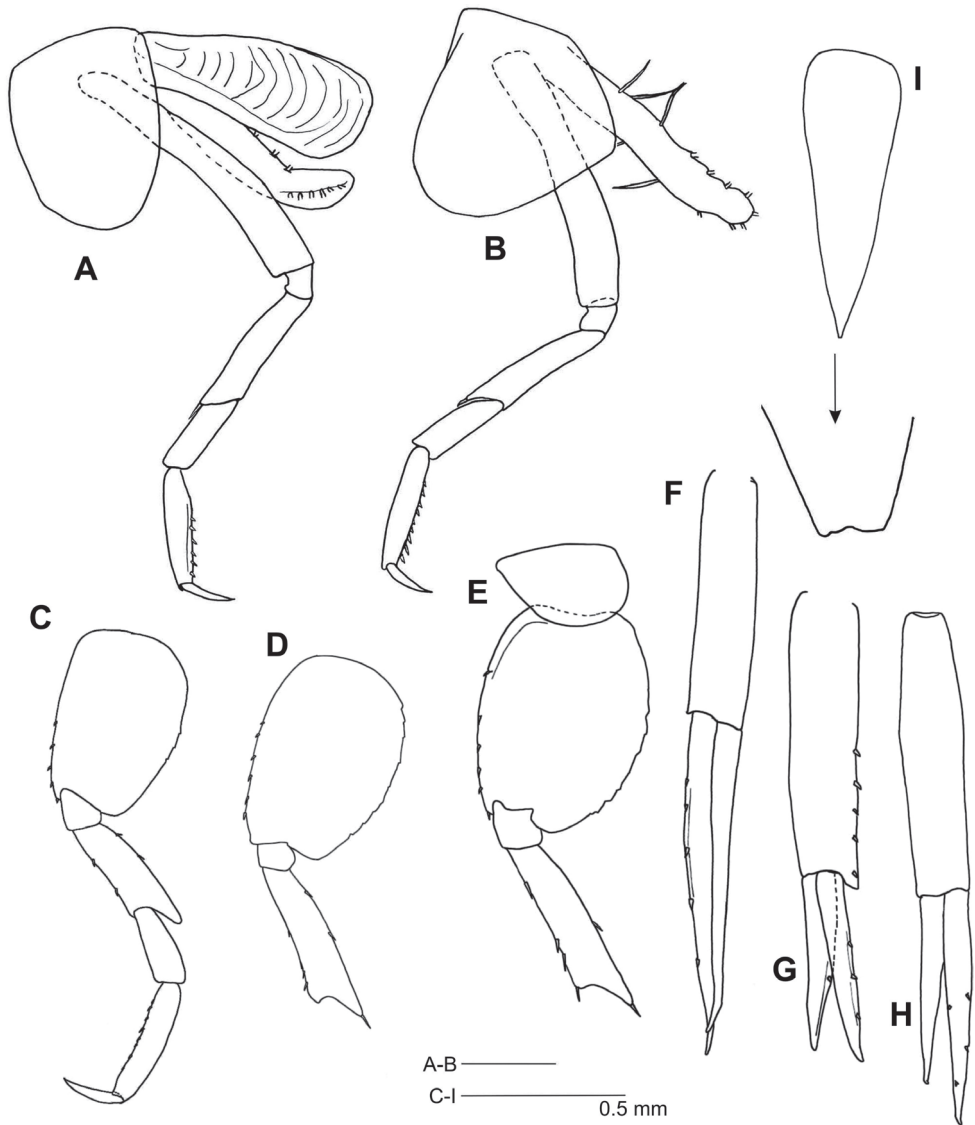
Figure 4. *Leucothoe kawesqari* sp. n. **A** Pereopod 3 **B** Pereopod 4 **C** Pereopod 5 **D** Pereopod 6 **E** pereopod 7 **F** Uropod 1 **G** Uropod 2 **H** Uropod 3 **I** Telson.

polychaetes *Platynereis australis* (Schmarda, 1861) and *Perinereis gualpensis* Jeldes, 1963, the bivalve *Aulacomya atra* (Molina, 1782) the decapod *Halicarcinus planatus* (Fabricius, 1775) and unidentified species of Echinodermata (Ophiuroidea and Psolidae), Porifera and Ascidiacea. These specimens of *L. kawesqari* were likely associated with or endocommensal associates of the Porifera and Ascidacea specimens withinin the sample, since the sampling method (scraping substrate) dislodges the samples and everything was sorted through at one time (White 2011, White and Reimer 2012).
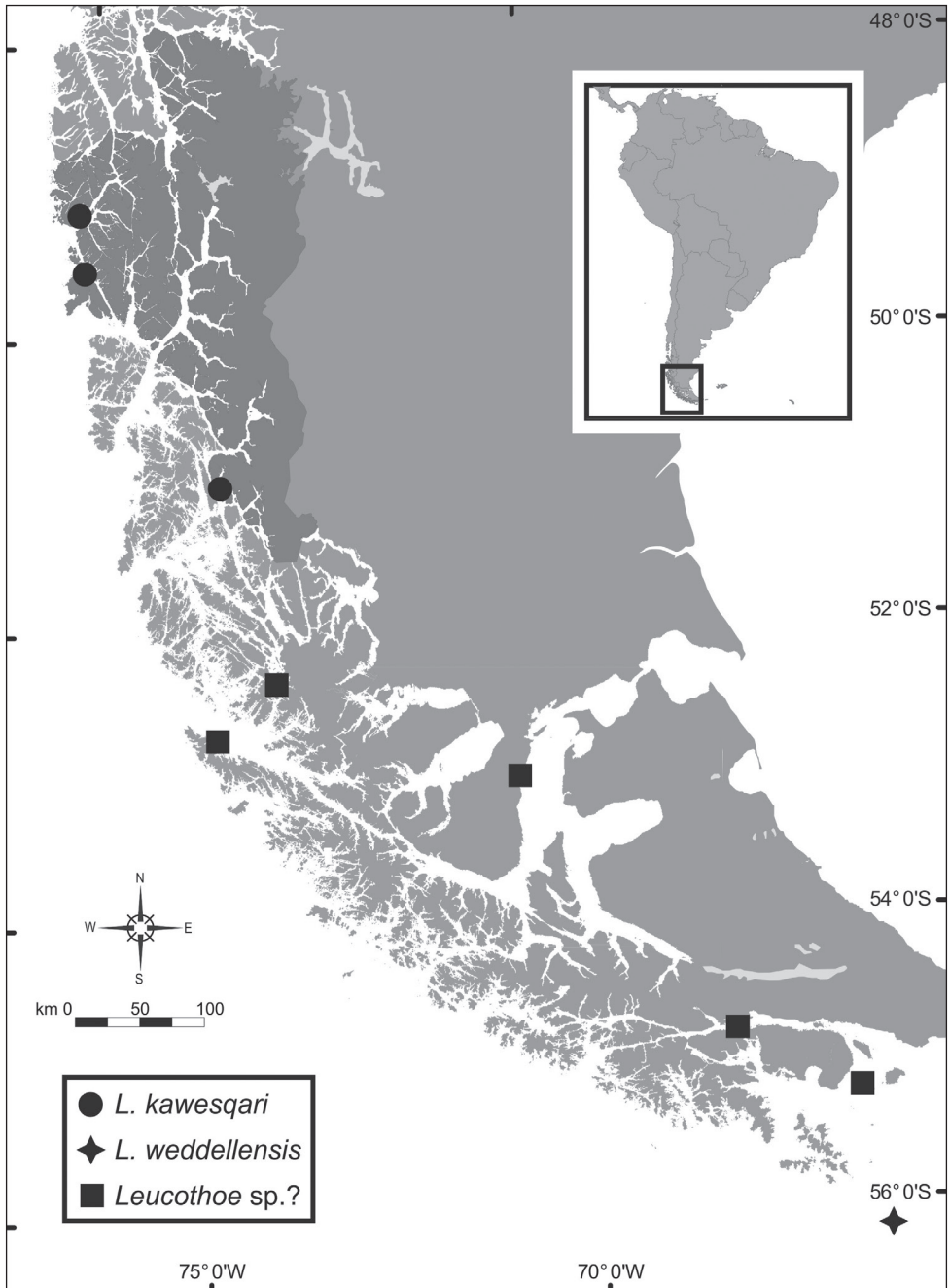
Figure 5. Location of the records of species of *Leucothoe* in the Magellan Region. BONP is shown overshadowed. *Leucothoe* sp.? corresponds to those in Schellemberg (1931) as *Leucothoe spinicarpa* which cannot be attributed to any known species (see text).

Discussion

The two species currently described for the Magellan Region have a well separated geographical distribution (Fig. 5): While the specimens of *L. kawesqari* come from the channels and fjords, *L. weddellensis* was found off shore, south of Tierra de Fuego and is distributed throughout the Antarctic seas (Krapp-Schickel and De Broyer 2014). Schellenberg (1931) reported *Leucothoe* in Cabo Valentina, Rio Seco, Strait of Magellan, Canal Beagle, and Isla Lennox. As mentioned above no illustration was provided, hence his records cannot be attributed to any described species. These locations lie between the distribution areas of the two known species. Otherwise, there are no more records of *Leucothoe* for the southeast coast of the Pacific. Further north, *Leucothoe panpulco* Barnard, 1961 is found in Acapulco and Panamá, and *Leucothoe alata* (Barnard, 1959) in California, with no overlap of distribution ranges of species of *Leucothoe* along the Pacific.

The geographical distribution of the species of *Leucothoe* studied by Krapp-Schickel and De Broyer (2014) and the data presented herein thus complete a latitudinal turnover of *Leucothoe* species along the west coast of the American continent, having from the north toward south, *L. kawesqari*, *L.* sp. and *L. weddellensis*. Nevertheless, large areas remain largely undersampled; future surveys in the East pacific including the Magellan region would reveal whether there are regions where species of the genus overlap, or a total latitudinal species turnover due to speciation through colonization.

Acknowledgments

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RESEARCH ARTICLE

A new species of *Auyantepuia* González-Sponga, 1978 (Scorpiones, Chactidae) from French Guiana

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Abstract

A new species of scorpion belonging to the genus *Auyantepuia* González-Sponga, 1978 (family Chactidae Pocock, 1893) is described on the basis of three specimens collected in a rainforest formation located in Saut Sabbat, South of Mana, French Guiana. This is the tenth species of the Guiano-Amazonian genus *Auyantepuia*, and the fifth reported from French Guiana.

Keywords

Scorpiones, *Auyantepuia*, new species, French Guiana

Introduction

In the present paper, a new species of *Auyantepuia* is described from a rainforest formation in French Guiana. The great diversity and endemism in the Guiana region has been previously discussed and evidence from scorpion biogeographic patterns has already been used to support the Guiana region as an important area of endemism (Lourenço 1986, 1991, 2001). The description of the new species raises to ten the number of species belonging to the genus *Auyantepuia* and confirms again the validity of this genus (Lourenço and Qi 2007) and the disrupted and relictual pattern of geographical distribution of the genus, which is confined to the Guiano-Amazon regions,

with a strong concentration of the species in the Guayana floristic province (Mori 1991). This also brings further confirmation to the very high levels of endemic species in the Guiana region.

Methods

Measurements and illustrations were made using a Motic DM143 digital stereo-microscope. Measurements follow Stahnke (1970) and are given in mm. Trichobothrial notations are those developed by Vachon (1974) and the morphological terminology mostly follows Hjelle (1990).

Taxonomic treatment

Family Chactidae Pocock, 1893

Genus *Auyantepuia* González-Sponga, 1978

Revised diagnosis for the genus. Scorpions of small size with a total length of 19 to 28 mm. General coloration reddish-brown to dark brown. Tegument smooth overall. Pedipalp chelal fingers very short, with trichobothria *db* and *esb* almost always at the same level; in some species these can be basal to trichobothrium *Et5*. Trichobothrial pattern of type *C*; neobothriotaxic 'majorante' (Vachon, 1974). Ventral aspect of metasomal segment V with strong granulations distally, which can form an arc.

Composition of the genus *Auyantepuia*

Auyantepuia scorzai (Dagert, 1957) (Venezuela)
Auyantepuia fravalae Lourenço, 1983 (French Guiana)
Auyantepuia gaillardi Lourenço, 1983 (French Guiana)
Auyantepuia sissomi Lourenço, 1983 (French Guiana)
Auyantepuia parvulus (Pocock, 1893) (Brazil)
Auyantepuia kelleri Lourenço, 1997 (French Guiana)
Auyantepuia mottai Lourenço & Araujo, 2004 (Brazil)
Auyantepuia amapaensis Lourenço & Qi, 2007 (Brazil)
Auyantepuia surinamensis Lourenço & Duhem, 2010 (Suriname)
***Auyantepuia laurae* sp. n.** (French Guiana)

Auyantepuia laurae sp. n.
http://zoobank.org/8CC80D99-F670-43AD-938B-B1508684206E
Figs 1–12, Table 1

Type material. One female holotype and two female paratypes. French Guiana, near Saut Sabbat, 50 km south of Mana and 50 km east of Saint-Laurent-du-Maroni, under wood log, I/2015 (E. Ythier & G. Roy). Deposited in the Muséum national d'Histoire naturelle (MNHN), Paris. Comparative material examined: *A. fravalae*, 1 male holotype (MNHN-RS-8505) and 1 female allotype (MNHN-RS-8506); *A. gaillardi*, 1 male holotype, 1 female allotype and 6 female paratypes (MNHN-RS-3311), 4 female paratypes (MNHN-RS-3307) and 1 male paratype (MNHN-RS-3326); *A. sissomi*, 1 female holotype (MNHN-RS-3304) and 1 female paratype (MNHN-RS-3309).

Etymology. The specific name refers to Laura Ythier, for her contribution to the collection of the new species.

Diagnosis. Small scorpions, 27.5 to 28.2 mm in total length. Coloration reddish-brown, with carapace, chelicerae, pedipalps and legs intensely marked with darker spots. Body and appendages weakly granulated or smooth; dorso-internal carina of chela inconspicuous; ventral posterior granulations on metasomal segment V weakly marked. Female pectines with 5-6 to 6-6 teeths; male unknown. Trichobothrial pattern of type C neobothriotaxic 'majorante'.

Description. based on female holotype and female paratypes.

Coloration. General coloration reddish-brown. Carapace reddish-yellow, intensely marked with brownish variegated spots around the ocular tubercle and on the anterior and posterior edges of the carapace; ocular tubercle darker, almost black. Tergites reddish-brown with confluent reddish-yellow spots, on the sides and the middle of tergites, without forming a longitudinal stripe. Metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots; vesicle reddish-yellow with basis of aculeus blackish and tip of aculeus reddish. Chelicerae yellowish, with variegated dark brown spots; fingers reddish-yellow with dark brown spots at their basis, reddish teeth. Pedipalps reddish-brown, with longitudinal dark brown spots. Legs yellowish, intensely marked with brownish spots. Venter and sternites yellowish to reddish-yellow; sternum reddish-yellow with darker spots; genital opercle reddish-yellow; pectines pale yellow.

Morphology. Carapace lustrous and acarinate, with some minute punctations; furrows shallow; anterior edge emarginate. Sternum pentagonal, wider than long. Tergites acarinate, almost smooth and shiny, with only minute granulations on their posterior edges. Pectinal tooth count 5-6 to 6-6, fulcra absent. Sternites smooth and shiny, VII acarinate; spiracles rounded in shape. Only metasomal segments IV and V longer than wide; metasomal tegument almost lustrous, without granulation, and with a few punctations; segment V with spinoid granulation ventrally, weakly marked. Carinae on segments I-V vestigial or absent; only dorso-lateral carinae are weakly marked on segments I to IV. Pedipalp femur with dorsal internal, dorsal external and ventral in-
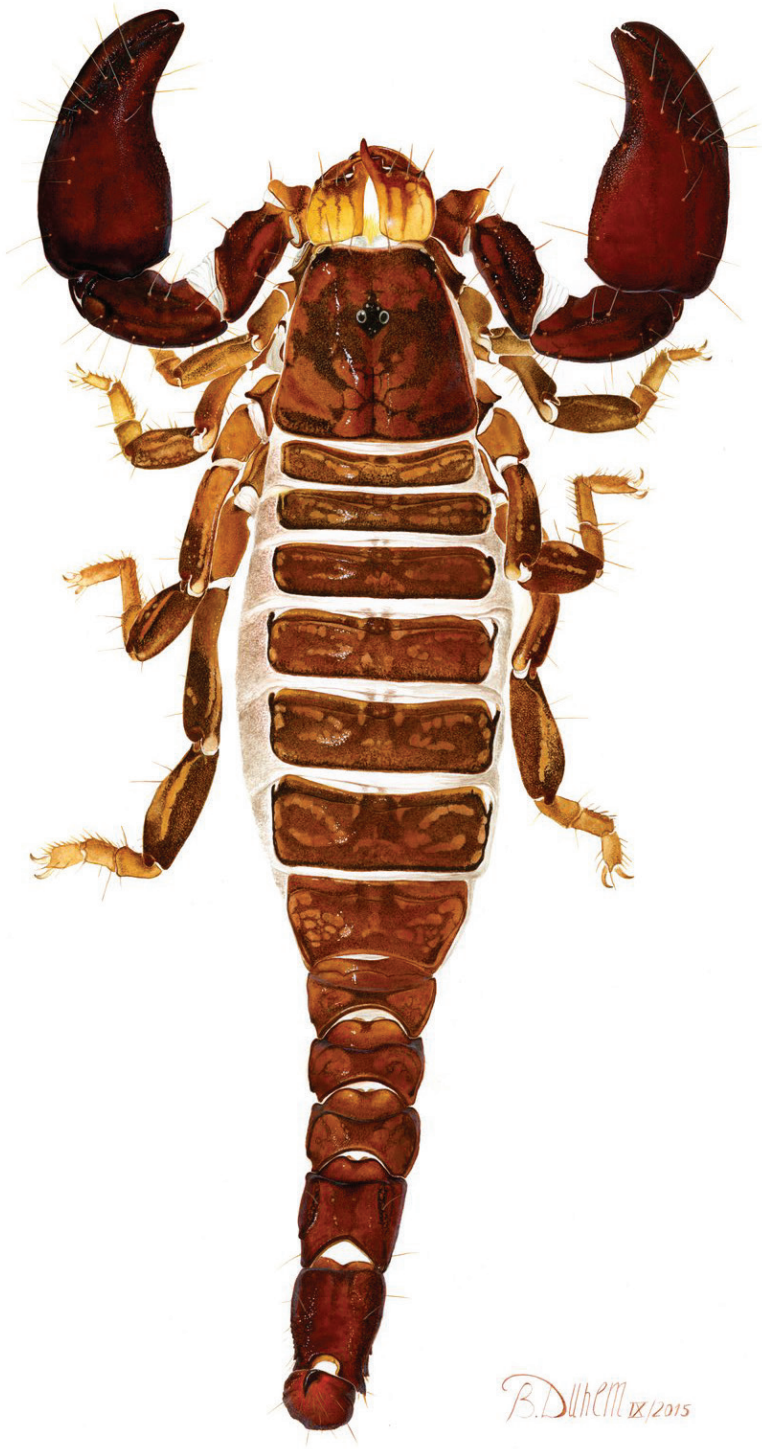
Figure 1. Habitus of *Auyantepuia laurae* sp. n., female holotype.
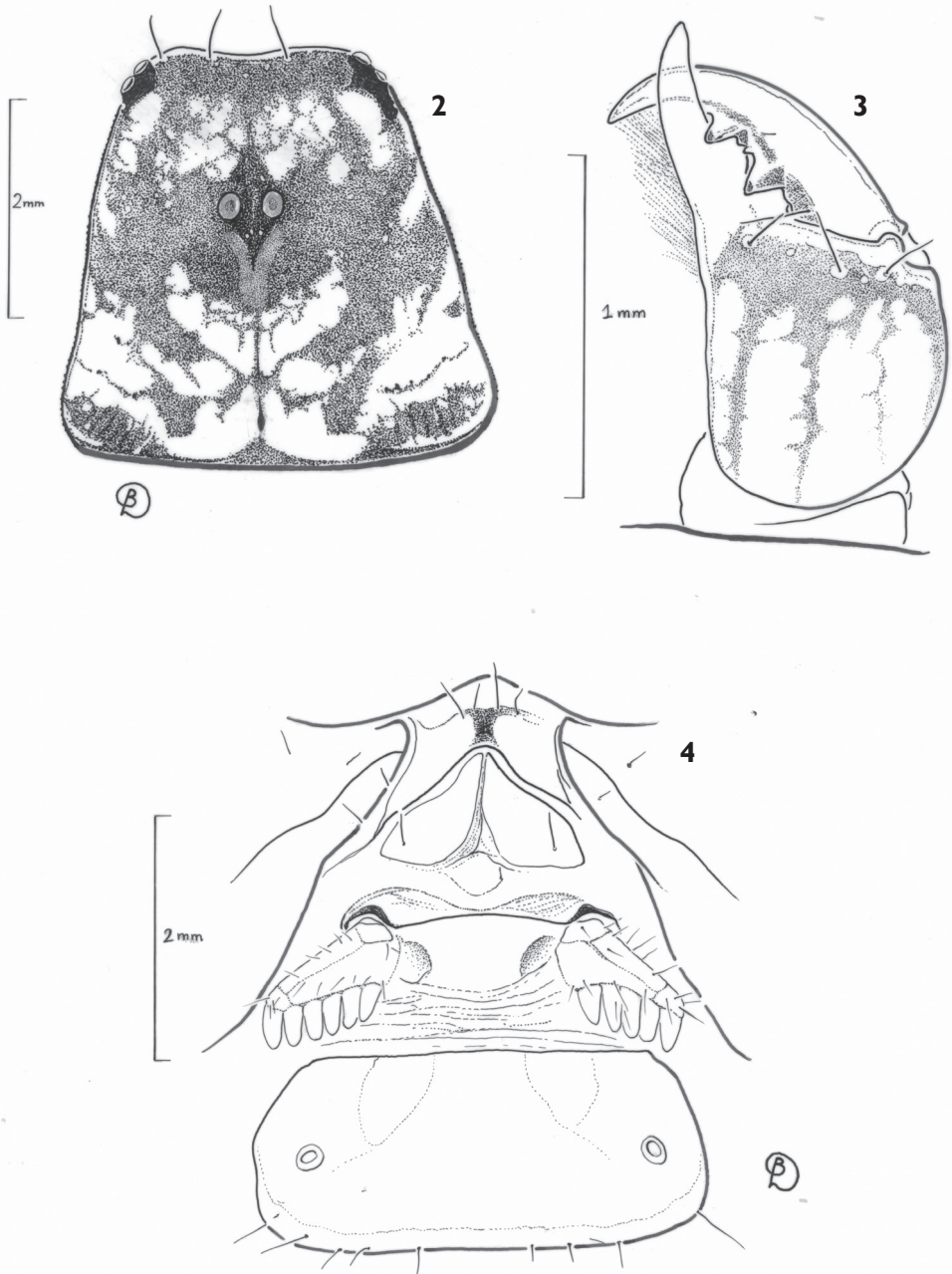
Figures 2–4. *Auyantepuia laurae* sp. n., female holotype. **2** Carapace, dorsal aspect **3** Right chelicera, dorsal aspect **4** Ventral aspect showing sternum, genital operculum, pectines and sternite III with spiracles.
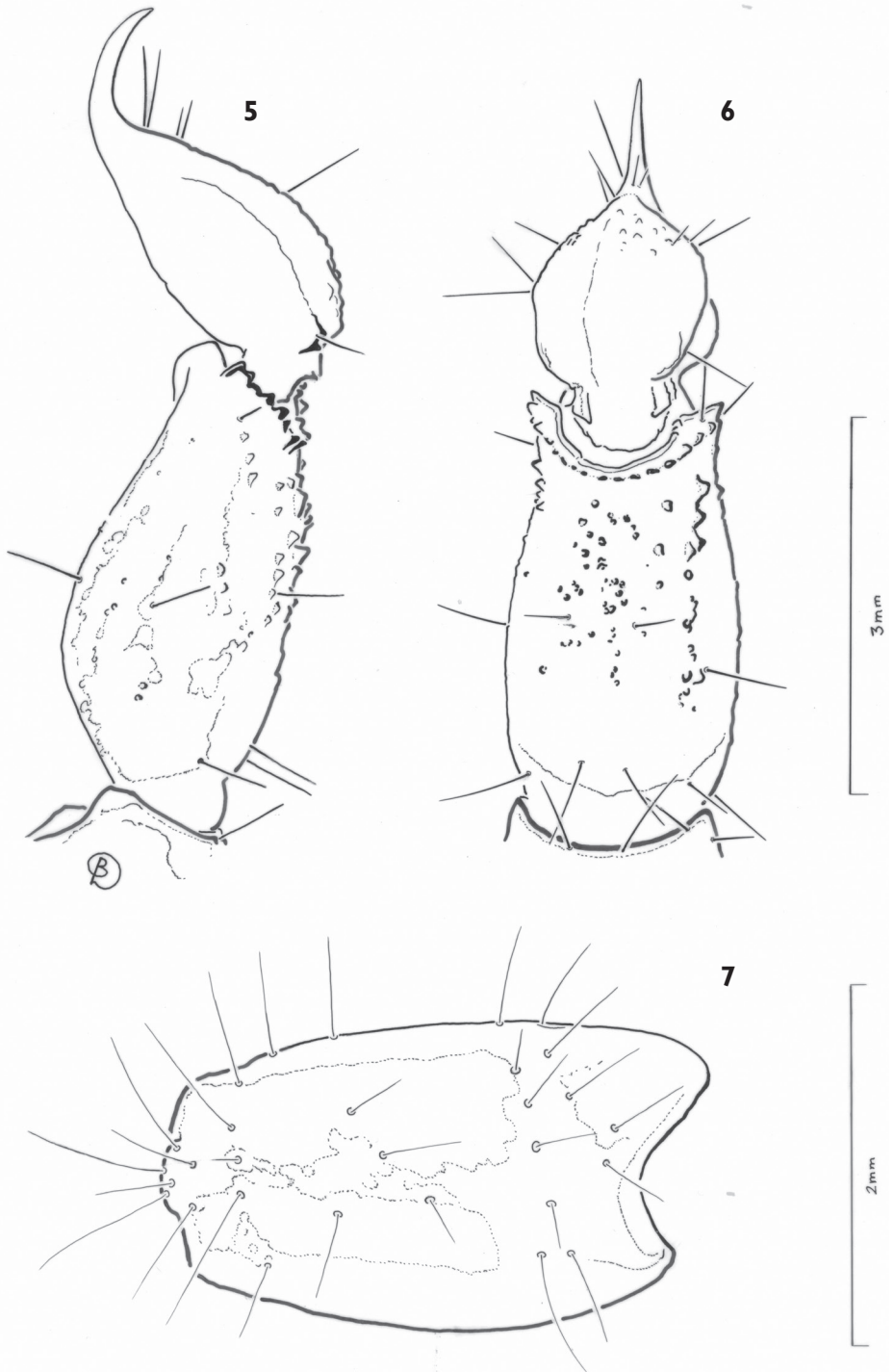
Figures 5–7. *Auyantepuia laurae* sp. n., female holotype. **5–6** Metasomal segment V and telson, lateral and ventral aspects **7** Patella, external aspect.
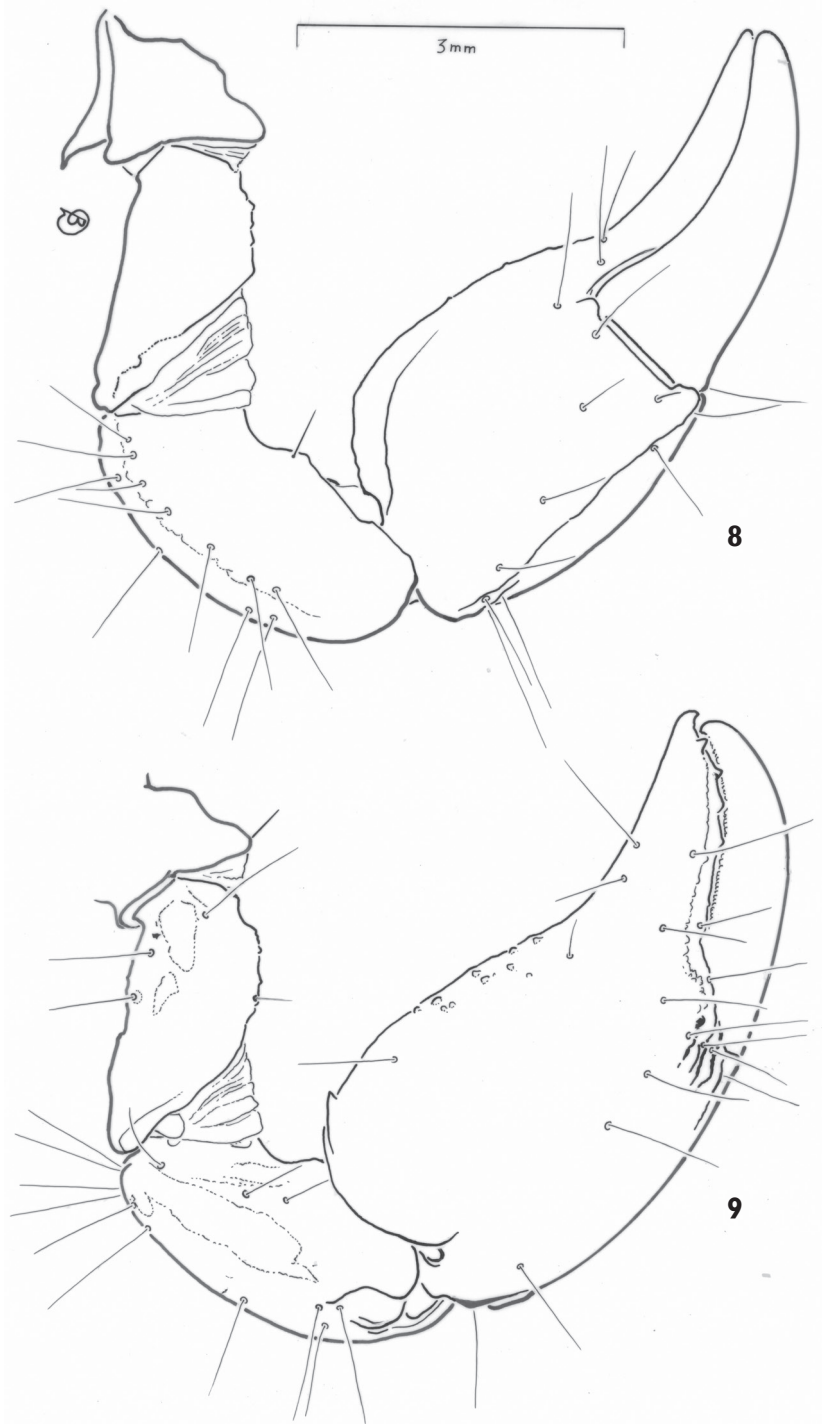
Figures 8–9. *Auyantepuia laurae* sp. n., female holotype. **8–9** Left (ventral view) and right (dorsal view) pedipalps, showing trichobothrial pattern.

Figure 10. *Auyantepuia laurae* sp. n., female holotype from Saut Sabbat, French Guiana, alive in the field.

Figure 11. Natural habitat of *Auyantepuia laurae* sp. n. in Saut Sabbat, French Guiana.
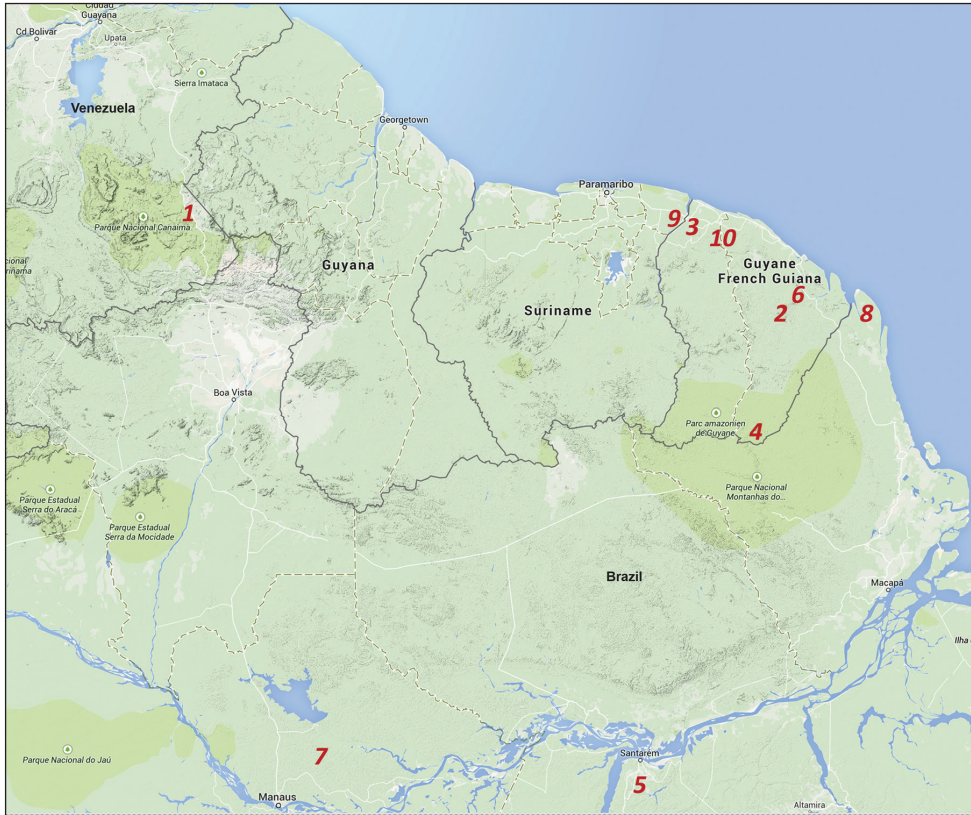
Figure 12. Records of *Auyantepuia* species in Guiano-Amazon regions, tropical South America: *A. scorzai* (**1**), *A. fravalae* (**2**), *A. gaillardi* (**3**), *A. sissomi* (**4**), *A. parvulus* (**5**), *A. kelleri* (**6**), *A. mottai* (**7**), *A. amapaensis* (**8**), *A. surinamensis* (**9**) and *A. laurae* sp. n. (**10**).

ternal carinae moderately marked; internal face weakly granular; other faces smooth. Patella smooth, with vestigial carinae. Chela weakly granulated, almost smooth, with dorso-internal carina weakly marked. Dentate margins on fixed and movable fingers with 6 rows of granules. Chelicerae with dentition typical of the family Chactidae (Vachon, 1963), and with dense setation ventrally and internally. Trichobothriotaxy of type C; neobothriotaxic 'majorante' (Vachon, 1974).

Relationships. *Auyantepuia laurae* sp. n. can be distinguished from other species of the genus *Auyantepuia* and, in particular, from the five species described from the Guiana region, by the following features:

- *A. gaillardi* Lourenço, 1983 (described from Saint-Laurent-du-Maroni, French Guiana): (i) metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots (all segments uniformly reddish in *A. gaillardi*), (ii) body, carapace,chelicerae, pedipalps and legs reddish-brown intensely marked with darker spots (uniform coloration without darker spots in *A. gaillardi*).

Table 1. Morphometric values (in mm) of *A. gaillardi* Lourenço, 1983 (female paratype), *A. surinamensis* Lourenço & Duhem, 2010 (female paratype), *A. sissomi* Lourenço, 1983 (female holotype), *A. fravalae* Lourenço, 1983 (female allotype) and *A. laurae* sp. n. (female holotype).

	A. gaillardi ♀	*A. surinamensis* ♀	*A. sissomi* ♀	*A. fravalae* ♀	*A. laurae* sp. n. ♀
Total length	26.9	20.8	26.2	28.6	28.2
Carapace:					
- length	4.1	3.6	3.6	4.3	4.0
- anterior width	2.7	2.3	2.4	2.9	2.1
- posterior width	4.1	3.7	3.6	4.8	4.6
Mesosoma length	7.5	6.0	9.8	9.0	10.5
Metasoma length	15.3	11.2	12.8	15.3	13.7
Metasomal segment I:					
- length	1.8	1.2	1.5	1.7	1.7
- width	2.6	2.2	2.4	2.3	2.6
Metasomal segment II:					
- length	2.1	1.4	1.6	1.8	1.8
- width	2.2	1.9	2.1	2.2	2.1
Metasomal segment III:					
- length	2.5	1.6	1.9	2.0	1.9
- width	2.2	1.8	2.1	2.1	2.0
Metasomal segment IV:					
- length	2.4	1.9	2.1	2.3	2.2
- width	2.0	1.7	1.9	2.0	2.0
Metasomal segment V:					
- length	3.4	2.9	3.0	3.8	3.4
- width	1.9	1.5	1.7	1.8	1.8
- depth	1.6	1.3	1.4	1.6	1.4
Telson:					
- length	3.1	2.2	2.1	2.8	2.7
- width	2.4	1.2	1.4	1.5	1.4
- depth	1.6	0.9	0.9	1.3	0.9
Pedipalp:					
- femur length	2.5	2.1	2.1	2.9	2.4
- femur width	1.2	1.2	1.1	1.3	1.0
- patella length	3.0	2.6	2.7	3.3	2.9
- patella width	1.6	1.3	1.3	1.6	1.4
- chela length	5.8	4.7	5.4	6.7	5.5
- chela width	2.2	1.7	2.0	2.4	2.0
- chela depth	3.1	2.0	2.3	2.9	2.8
Movable finger length	3.0	2.3	2.6	3.4	3.0

- *A. surinamensis* Lourenço & Duhem, 2010 (described from Albina/Moengo, Suriname): (i) metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots (all segments reddish uniformely and intensely marked with brownish spots in *A. surinamensis*), (ii) size 27.5–28.2 mm (19.0–20.8 mm in *A. surinamensis*).

- *A. kelleri* Lourenço, 1997 (described from Cacao, French Guiana): (i) metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots (all segments uniformely dark reddish in *A. kelleri*), (ii) pedipalps intensely marked with dark brown spots (weakly marked in *A. kelleri*), (iii) occular tubercle darker, almost black (clear in *A. kelleri*).

- *A. fravalae* Lourenço, 1983 (described from Saut Pararé, French Guiana): (i) metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots (brownish spots on lateral and ventral sides of segments I to V and ventral side of segments I to II well pigmented in *A. fravalae*), (ii) pedipalps with chelae weakly granulated, almost smooth (moderately to strongly granulated in *A. fravalae*), (iii) female pectines with 5–6 to 6–6 teeth (8–8 in *A. fravalae*).

- *A. sissomi* Lourenço, 1983 (described from Oyapok, French Guiana): (i) metasomal segments reddish-yellow, marked with variegated brownish spots on lateral and dorsal sides of segments I to V and on ventral side of segments IV and V; ventral side of segments I to III yellowish, without spots (only ventral side of segments I to II *yellowish in A. sissomi*), (ii) pedipalps with chelae weakly granulated, almost smooth (moderately to strongly granulated in *A. sissomi*), (iii) tergites reddish-brown with confluent reddish-yellow spots, on the sides and the middle of tergites, without forming a longitudinal stripe (yellow spots on the middle of tergites forming a longitudinal stripe dividing the tergites in *A. sissomi*), (iv) general coloration reddish-brown (yellowish in *A. sissomi*).

Key to the species of *Auyantepuia* described from the Guiana region

1   Pedipalps with chelae weakly granulated, almost smooth **2**
–   Pedipalps with chelae moderately to strongly granulated............................**5**
2   Ventral side of metasomal segments I to III yellowish, without spots............
         ....***A. laurae* sp. n.**
–   Ventral side of all metasomal segments well pigmented, brownish to dark reddish...**3**
3   Body, pedipalps, legs and chelicerae without variegated brownish spots........
         ....***A. gaillardi***
–   Body, pedipalps, legs and chelicerae marked with variegated brownish spots... **4**

4		Occular tubercle dark, almost black .... *A. surinamensis*
–		Occular tubercle clear .... *A. kelleri*
5		Ventral side of metasomal segments I to II yellowish, without spots ....
		....*A. sissomi*
–		Ventral side of all metasomal segments well pigmented, brownish to dark
		reddish..*A. fravalae*

Acknowledgements

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RESEARCH ARTICLE

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Rediscovery of *Eremobittacus spinulatus* Byers (Mecoptera, Bittacidae) in Mexico, with description of the female and comments on sexual dimorphism and potential mimicry

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Abstract

The female of *Eremobittacus spinulatus* Byers, 1997 is described for the first time. A key to the two species known of this genus endemic to Mexico is provided, and species distribution is illustrated. A case is made for adults of *Eremobittacus* to be sexually dimorphic, which appears to be an exceptional occurrence in Bittacidae. It is claimed that *E. spinulatus* habitus has a wasp-like appearance, which may potentially depict a case of mimicry.

Keywords

Hangingfly, sexually dimorphic, tropical dry forest, taxonomy, key, mimicry

Introduction

Eremobittacus was erected by Byers (1997) with *E. spinulatus* Byers as type species. This genus is endemic to Mexico and includes a second species, *E. sodalium* (Byers 2011); each described from a single male specimen. Immature stages and females have remained unknown, because of an apparent rarity of these species. Byers (1997) comments that he desired for additional specimens to achieve a more complete description of the genus, as well as an accurate phylogenetic placement. Byers and other entomologists visited again *E. spinulatus* type locality and were unable to find additional specimens.

Eremobittacus shares several traits with the widespread *Bittacus*, as well as with *Harpobittacus*, an endemic of Australia. Alignment of longitudinal veins in three slightly pigmented columns, wing venation, body microsculpture, particularly of hind femora, and coloration, were used as diagnostic traits for the genus. However, after *E. sodalium* was described, the genus diagnosis was slightly modified: vein A1 long, with its distal end beyond origin of M, basitarsus of hind leg almost the same length of tarsomeres II and III together, noticeable thickening of hind femur, basistyle short and bulging, dististyle with setae only on margin, cerci very short and aedeagus uncoiled (Byers 2011).

In Bittacidae, most diagnostic characters used for species differentiation are found in the male genitalia. From species descriptions (e.g., Byers 1996, 2004) it becomes evident that modifications in shape and size of abdominal sclerites of the female might also help in species identification. Nonetheless, we have little female genitalia diagnostic information available for other bittacid species. Within Bittacidae, males are readily distinguished from females, as the former display a modified terminalia, bearing a pair of epiandric appendages, an aedeagus with varying degrees of lengthening (called penisfilum when elongated, either coiled or uncoiled), a pair of reduced gonostyli (dististyles), a pair of pheromone producing eversible sacs between tergites VI and VII, and VII and VIII, as well as a characteristic flight pattern (Thornhill 1984, Byers 1996, Gao and Hua 2013). Yet, typically general appearance of both sexes is very similar. For this reason, an apparent case of sexual dimorphism appears worth describing. Moreover, a striking wasp-like appearance of the adult habitus, most remarkable in the male, also is worth noting.

Methods

During examination of specimens at the National Collection of Insects of the Institute of Biology (CNIN-UNAM), a series of six specimens of *E. spinulatus* immediately stood out because of the noticeable enlargement of the hind femora of males (less noticeable in females). Half of the specimens were female, allowing the first description of a female of this genus. Also, additional distribution data are presented for the species and observations on sexual dimorphism are discussed. Male and female specimens were dissected with a previous rehydration and terminalia were studied after clearing in 10% KOH at room temperature. Measurements were taken with a digital caliper and

ranges are presented in mm with average in parentheses. Photographs were taken with an automontage system in a Zeiss Axio Zoom V16 stereomicroscope, while observations were made on a Zeiss Discovery V8 stereomicroscope.

Results

Eremobittacus spinulatus Byers
Figs 1–6

Description. *Male* (Fig. 1; n = 3, pinned). Forewing length 13.0–13.3 (13.2); forewing width 2.7–3.2 (3.0); antenna length 5.9-6.6 (6.2); hind femur length 6.0-6.4 (6.2); hind femur width 1.2–1.3 (1.25). Original description in Byers (1997).

Female (Fig. 2; n = 3, pinned). Forewing length 12.2–13.1(12.7); forewing width 3.0; antennae length 6.0–6.5 (6.2); hind femur length 5.1-5.4 (5.2); hind femur width 0.63–0.68 (0.65). General appearance similar to male, particularly in coloration and general body proportions, however hind femora appear narrower than in male (Fig. 1).

Abdomen (Fig. 4). Terga and pleura dark brown to entirely black, with dark short setae; VIII sternite fused with the subgenital plate, dorsally separated from tergites VIII and IX by an incision; stigma of segment VIII above a concavity in the sternite; subgenital plate with about 30 conspicuous dark spine-like setae, with an oblique plate connected with the 9th tergite; short non segmented cerci reaching about half the length of supra anal plate (or XI tergite).

Legs (Fig. 2). First and second pair of legs pale brown, with darkened areas at joints; hind legs modified, basal three fourths of femur blackish brown, distal fourth pale brown, slightly widened at median; widening about half that in males (Fig. 1), conveying sexual dimorphism; also, femur spine-like setae less developed than in males.

Intraspecific variation. In the original description by Byers (1997, fig. 11), the male epiandric appendix is illustrated with a slight median protuberance, dorsally; however, the protuberance is inconspicuous in the specimens examined (Fig. 3). Furthermore, a ventral view of the basistilum (*bs*) was not included in the original description (herein shown in Fig. 5) and its setation was illustrated only partially (herein shown in Figs 3, 5 and 6). One of the main diagnostic traits proposed for this species is a pattern of three transverse veins surrounded by a darkened region in both fore and hindwing; conversely, this feature was found variable, as in some specimens the pattern is diffuse or dimly visible. The wing longitudinal veins also display a certain degree of variation, for example, in number of veins between R_2 and R_3, R_3 and R_4, and between R_5 and M_1, as well as in number of *Pcv* veins under the pterostigma.

Material examined. Mexico, Oaxaca, 26 km SE Cuicatlán, 17°37'02.09"N, 96°55'23.52"W, 1080 m, 16-X-1998, M.A. Morales, 1♀; same data except E. Ramirez collector, 1♀; Oaxaca, 25 km SE Cuicatlán, 17°37'16.38"N, 96°55'10.02"W, 1000 m, 17-X-1998, F. Noguera, 1♀; Oaxaca, 26 km SSE Cuicatlán, 17°36'9.88"N, 96°55'39.2"W, 1080 m, 16-X-1998, E. Ramírez, 1♂; same data except M.A. Morales, 1♂; same data except 18-X1998, 1♂.
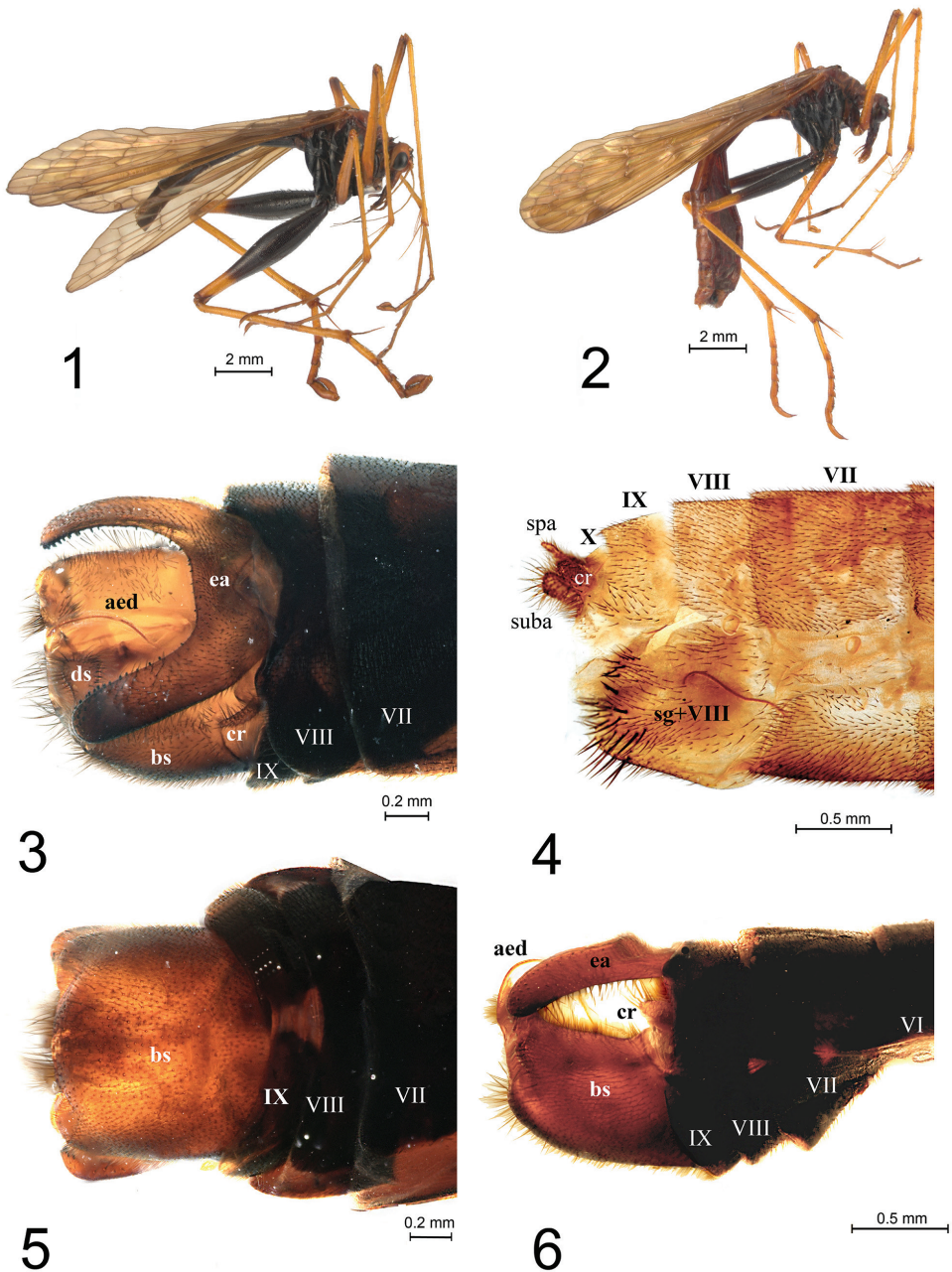
Figures 1–6. *Eremobittacus spinulatus* Byers. **1** Male habitus, lateral view **2** Female habitus, lateral view **3** Male genitalia, dorsal view **4** Female genitalia, lateral view **5** Male genitalia, ventral view **6** Same, lateral view. Abbreviations: aed, aedeagus; bs, basistyle; cr, cerci; ds, dististyle; ea, epiandrial appendage; spa, supra-anal plate (= XI tergum); suba, sub-anal plate (= XI sternum); sg, subgenital plate; roman number denotes abdominal segments.
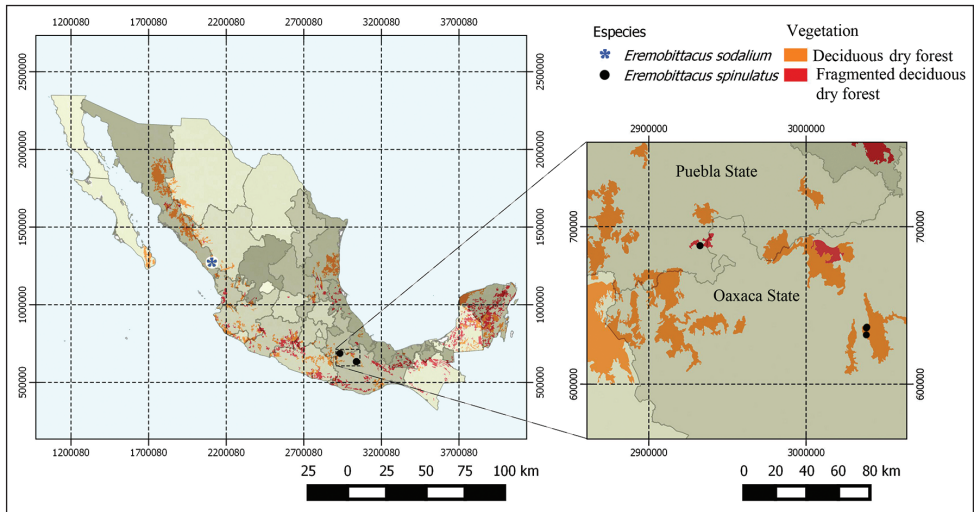
Figure 7. Present distribution of the genus *Eremobittacus* in Mexico.

Key to the species of *Eremobittacus* Byers

1   Cuticle of whole body and hind femora with spiny surface, contrasting coloration present (black and orange), crossveins between *R* and *M* aligned transversely, epiandrial appendage subequal in thickness; Oaxaca and Puebla (Mexico) .... *E. spinulatus* **Byers, 1997**
–   Cuticle of whole body and hind femora without spiny surface, contrasting coloration absent, crossveins between *R* and *M* not aligned transversely, epiandrial appendage noticeably reducing in thickness apically; Sinaloa (Mexico) ....
.... *E. sodalium* **Byers, 2011**

Notes on distribution (Fig. 7). *Eremobittacus spinulatus* was known only from Puebla state (near Petlalcingo), east central Mexico. Herein, records for the state of Oaxaca (southeastern Mexico) are presented for the first time, increasing its distribution range in about 125 km. Both localities are within tropical dry forest, as is the type locality of *E. sodalium* in the state of Sinaloa. This ecosystem in Mexico is characterized by its high biological diversity and also by a high degree of endemicity (Dirzo and Ceballos 2010, Noguera et al. 2012).

Discussion

Eremobittacus (exemplified here with *E. spinulatus*) appears to depart from a typical hangingfly habitus (i.e., similar to a cranefly, with long slender legs of subequal shape; Figs 1–2), the generalized condition in Bittacidae. From here, we believe two points should

be made. First, there is a distinct sexual dimorphism, evidenced by thicker femora of hind legs in males, and to a less degree, a denser spiniform setation in male hind femora, as compared to females. Sexual dimorphism, to our knowledge, had not been recorded before for a bittacid. In itself, this phenomenon requires further study (e.g., morphometrics, behavior) for a more accurate description, as well as a possible explanation (e.g., sexual selection). According to Byers and Thornhill (1983), visual signals (e.g., wing and body movements) are important in close-range sexual interactions in *Panorpa* and bittacids, so a visual interaction between sexes is not disparate as a first working hypothesis that may explain a selective force leading to dimorphism in these species (e.g., female choice of males with thicker femora). This could be part of the customary nuptial gift of a prey item offered by males to females, in which females discriminate against males with unpalatable or small prey by flying away (Byers and Thornhill 1983).

Second, we suggest that the genus habitus is wasp-like (Figs 1, 2), similar to a sphecid (e.g., *Chalybion*, *Sceliphron*) or crabronid wasp. Again, this working hypothesis would require observation in nature in order to test whether behavior of the species agrees with such wasp-like resemblance (i.e., a case of mimicry). If in nature, *Eremobittacus* does not only behave as a typical hangingfly (e.g., a hanging food capturing strategy), but spends time capturing food in movement, as many bittacids do (Byers and Thornhill 1983), a wasplike, mimicry hypothesis would be supported. Although web-building spiders are frequent predators of Panorpidae and Bittacidae, they are also eaten by damselflies, robber flies, assassin bugs, and roving spiders (Byers and Thornhill 1983), some of which most likely would be discouraged by a wasp-looking potential prey (e.g., wasps have strong mandibles and sting). Recently, a case of mimicry has been made in fossil mecopterans (Wang et al. 2012). They mention a leaf mimesis of the hangingfly *Juracimbrophlebia ginkgofolia* Wang et al. (Cimbrophlebiidae) on a member of the Ginkgoales or ginkgos, *Yimaia capituliformis* (Yimaiaceae), from the Middle Jurassic of Mongolia. The insect wings resembled leaves in order to avoid predators (crypsis), or perhaps the insect provided an antiherbivore function for its plant hosts (mutualism). In *Eremobittacus*, a body robust and compact, with a slender abdomen attached to the thorax, a hind femur longer (and thicker) than other legs, and a potential warning coloration of the body, black and orange (as occurs in some wasps), may for the meantime represent a working hypothesis for another case of mimicry in the Mecoptera.

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RESEARCH ARTICLE

Conidarnes, a new oriental genus of Sycophaginae (Hymenoptera, Agaonidae) associated with *Ficus* section *Conosycea* (Moraceae)

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Abstract

The sycophagines are strictly associated with two subgenera of *Ficus* L. (Moraceae), namely *Sycomorus* and *Urostigma*. They mostly oviposit through the fig wall and lay their eggs within the fig flowers, being either gall-makers or parasitoids of other fig wasps. In this contribution, a new genus of Sycophaginae, *Conidarnes* Farache & Rasplus, **gen. n.**, is described with seven new species: *Conidarnes achterbergi* Farache & Rasplus, **sp. n.**; *Conidarnes bergi* Farache & Rasplus, **sp. n.**; *Conidarnes laevis* Farache & Rasplus, **sp. n.**; *Conidarnes santineloi* Farache & Rasplus, **sp. n.**; *Conidarnes subtectae* Farache & Rasplus, **sp. n.**; *Conidarnes sulcata* Farache & Rasplus, **sp. n.**; and *Conidarnes sumatranae* Farache & Rasplus, **sp. n.** Illustrations, morphological diagnoses, dichotomous keys and multi-entry online keys to species are provided. *Conidarnes* species strictly occur in the oriental region, and their distribution does not overlap with the distribution of the two other genera belonging to the same clade. Due to their relative rarity, we encourage extensive sampling of *Conosycea* figs to improve our knowledge of the genus.

Keywords

Introduction

Recent phylogenetic analyses of the Chalcidoidea have retrieved Sycophaginae as sister to the pollinating Agaoninae (Heraty et al. 2013). Consequently, the family Agaonidae is now subdivided into two subfamilies: the Agaoninae and the Sycophaginae. The Agaoninae have established a very specialized relationship with *Ficus* L. (Moraceae) (Cook and Rasplus 2003). These wasps are the main pollinators of fig trees and are capable of entering the fig inflorescences through a small pore, called the ostiole. Once inside the figs, these wasps pollinate and lay their eggs in some pistillate flowers (Galil and Eisikowitch 1968). The Sycophaginae are non-pollinating fig wasps (NPFW) that are strictly associated with two subgenera of *Ficus* (Moraceae), namely *Sycomorus* and *Urostigma*. They mostly oviposit through the fig wall and lay their eggs inside the figs within the flowers, being either gall-makers or parasitoids of other fig wasps (Bouček 1988; Cruaud et al. 2011b; Elias et al. 2008). A few species (a small species-group of Afrotropical *Sycophaga* Westwood, Cruaud et al. 2011b) enter the fig through the ostiole (Galil et al. 1970).

There are about 60 described species of Sycophaginae that occur in all tropical and subtropical regions. The subfamily Sycophaginae was retrieved as a monophyletic assemblage and divided into three main clades each of which may warrant tribal status (Cruaud et al. 2011a):

1) A first clade—sister to the remaining Sycophaginae—that only includes *Eukoebelea* Ashmead species associated with *Ficus* subsection *Malvanthera* in Australasia.
2) A clade that includes species of large and early gallmakers, belonging to three genera: i) the Australasian genus *Pseudidarnes* Girault associated with *Ficus* subsection *Malvanthera*, ii) the Neotropical *Anidarnes* Bouček associated with *Ficus* section *Americana*, and iii) a few species associated with *Ficus* section *Conosycea* that cannot be placed in any existing genus which requires the establishment of the new genus described here.
3) Sister to the previous clade, a highly diversified clade composed of the New World *Idarnes* Walker associated with *Ficus* section *Americana*, and the Old World *Sycophaga* mostly associated with *Ficus* subgenus *Sycomorus*, but also including two species associated with *Ficus* subgenus *Urostigma* section *Urostigma*.

We have recently reviewed the genera *Anidarnes* and *Pseudidarnes* with the description of nine new *Anidarnes* and six new *Pseudidarnes* species (Farache et al. 2013; Farache and Rasplus 2014). In this paper, we propose the establishment of *Conidarnes*, a new oriental genus of Sycophaginae, and describe seven new species mostly sampled from figs of the large strangling fig trees (*Conosycea*) that occur in the dipterocarp rainforests of the oriental region. We also provide illustrations, morphological diagnoses, dichotomous keys, and multi-entry online keys to species.

Methods

Specimen handling and imaging follow Farache and Rasplus (2014). Geographical coordinates and altitudes were mostly estimated using label information. Morphological terminology follows Gibson (1997), and the HAO (Hymenoptera Anatomy Ontology) portal (Yoder et al. 2010). Species descriptions were assembled in DELTA (Dallwitz 1980). A list of characters and character states used to describe the species can be found in Suppl. material 1. Characters included in this list were matched with HAO portal codes. This may help readers to better understand the anatomical structures we used for description. The sections dealing with the material examined were prepared using AUTOMATEX (Brown 2013). Multi-entry identification keys were built using LUCID®, and are available at http://www.figweb.org.

Images were produced with a Leica M16 lens and a JVC KY-75U 3CCD digital camera. Cartograph v5.6.0 (Microvision, Evry, France) software was used for focus stacking.

Type and specimen depositories, and their respective curators are:

CBGP France, Montpellier. Centre de Biologie pour la Gestion des Populations (Emmanuelle Artige).
RMNH Netherlands, Leiden, Naturalis Biodiversity Centre (Frédérique Bakker).
RPSP Brazil, São Paulo, Ribeirão Preto, Universidade de São Paulo (Eduardo A. B. Almeida).
SAMC South Africa, Cape Town, Iziko South African Museum (Simon van Noort).

Results

Conidarnes Farache & Rasplus, gen. n.
http://zoobank.org/F3DA3DE4-65DC-4706-B53A-9694E571447F

Type species. *Conidarnes santineloi* Farache & Rasplus, sp. n.

Diagnosis. Antennae with 13–14 antennomeres (one or two anelli), including a stub or nipple-like terminal flagellomere. Funicular segments slightly longer than wide to transverse. Antennae inserted at the middle line of compound eyes or below. Toruli contiguous. Clypeal margin bilobed. Malar sulcus absent. Petiole very short, transverse. Ovipositor sheaths without a median constriction and depigmentation.

Generic description. *Females. Size and colour.* Body length 1.5–4.0 mm. Length of the ovipositor sheaths 0.4–6.4 mm. Body colour variable. Antennae mostly yellow, sometimes with orange or brown tinges. Head and mesosoma brown to black, usually with green, blue and orange metallic lustre. Legs yellow to brown. Coxae sometimes concolorous with mesosoma. Wings hyaline, sometimes medially infuscate in males. Metasoma usually brown black, sometimes yellow.

Head. Antenna with 13 or 14 antennomeres (including a stub or nipple-like terminal antennomere), usually with two anelli but sometimes with a single anellus (antennal formula 11263 or 11163). Terminal antennomere (*i.e.* a nipple-like thirteenth or fourteenth antennomere) sometimes conspicuous. Funicular segments slightly longer than wide to transverse. Face sculpture usually reticulate, sometimes slightly engraved. Upper face sometimes smooth. Antennae inserted at the middle line of compound eyes or below. Toruli contiguous, distance between toruli always smaller than one torulus diameter. Clypeal margin bilobed. Malar sulcus absent.

Mesosoma. Pronotum and mesonotum sculpture variable. Pronotum longer than high in lateral view. Notauli usually complete, but incomplete in *Conidarnes laevis* sp. n. Mesoscutellar-axillar complex with straight or incurved axillular grooves and transverse frenal sulcus, forming a square mesoscutellum (an apomorphy of Sycophaginae). Mesoscutellum trapezoidal, wider near frenal sulcus and narrower near transscutal articulation. Propodeum transverse. Wings with short and sparse pilosity. Postmarginal vein inconspicuous, stub-like. Marginal vein at least as long as stigmal vein.

Metasoma. Petiolate, petiole very short, transverse. Margin of eighth gastral tergite deeply sinuate, A-like, with thumbnail-like medial flap (epipygium) and with a peg-like cercus arising from the membrane on either side of the epipygium (apomorphy of Sycophaginae). Length of the ovipositor sheaths varying from 0.3× (about as long as the hind tibia) to nearly twice as long as body. Ovipositor sheaths without a median constriction and depigmentation.

Males. Similar to females but usually slender and shorter. Exhibiting different coloration, the mesosoma sometimes mostly yellow. Wings sometimes medially infuscate.

Etymology. The generic name is masculine and derived from *Idarnes* Walker, 1843, in the same manner than other sycophagine genera (*Pseudidarnes* Girault, 1927 and *Anidarnes* Bouček, 1993) and is associated to the prefix *Con* used for *Conosycea*, the host plant section of the included species. The origins of the name *Idarnes* were discussed in Farache et al. (2013).

Key to species

1   Notauli incomplete (Fig. 6D). Upper face smooth and lower face reticulate (Fig. 6C). *Ficus kerkhoveni* .... ***laevis* sp. n.**
–   Notauli complete. Face entirely reticulate **2**
2   Sculpture of mesoscutum and mesoscutellum mostly smooth, lateral lobes of mesoscutum engraved reticulate (Fig. 12D). Scrobe with a median longitudinal sulcus, extending from median ocellus to interantennal area (Fig. 12C). *Ficus altissima* ....***sulcata* sp. n**
–   Sculpture of mesoscutum and mesoscutellum reticulate, lateral lobes of mesoscutum reticulate. Scrobe without a median longitudinal sulcus **3**
3   Antenna with one anellus (Fig. 7B). Funicular segments mostly transverse (Fig. 7B). *Ficus pallescens* ....***santineloi* sp. n.**

–   Antenna with two anelli. Funicular segments mostly as long as wide or slightly longer than wide. **4**

4   Ovipositor approximately 1.5× as long as body (Fig. 1A). Metasoma ventrally yellow and dorsally dark brown (Fig. 1A) ....***achterbergi* sp. n.**

–   Ovipositor as long as body or shorter. Coloration of metasoma homogeneously brown **5**

5   Ovipositor nearly as long as body (Fig. 10A). Supraclypeal area wide (Fig. 10D). *Ficus subtecta* ....***subtectae* sp. n.**

–   Ovipositor nearly 0.5× as long as body (Figs. 3A, 14A). Supraclypeal area narrow (Figs 3D, 14C) **6**

6   Antennae inserted near the middle line of compound eyes (Fig. 14C). Supraclypeal area higher than clypeus (Fig. 14C). Propodeum with a reticulate median line, slightly striate, and wider near the anterior margin of the propodeum (Fig. 15D). *Ficus sumatrana* ....***sumatranae* sp. n.**

–   Antennae inserted at the lower line of compound eyes (Fig. 3D). Supraclypeal area shorter than clypeus (Fig. 3D). Propodeum without a well-established median line (Fig. 4B). *Ficus involucrata* ....***bergi* sp. n.**

Species descriptions

Conidarnes achterbergi Farache & Rasplus, sp. n.
http://zoobank.org/51232815-C2ED-4933-9561-0DD85A5AA95D
Figs 1, 2

Holotype. ♀: **MALAYSIA: SE. Sabah:** nr. Danum Valley Field C. El. C., 4.96° 117.69°, 400m, 21–25.III.1978, v. Achterberg C, Malaise trap 7 (CBGP).

Diagnosis. Metasoma ventrally yellow, dorsally dark brown. Antenna with two anelli. Antennae inserted at the lower line of compound eyes. Mesoscutum and mesoscutellum sculpture reticulate. Length of the ovipositor sheaths 1.7× body length.

Description. *Female. Size and colour.* Body length 3.8 mm. Length of the ovipositor sheaths 6.4 mm. Antennae yellow orange. Head and mesosoma with metallic lustre, mostly green and blue. Head dorsally more orange. Legs yellow. Metasoma ventrally yellow, dorsally dark brown.

Head. Scape 4.8× as long as wide. Antenna with two anelli. Proximal anellus longer than distal anellus. Funicular segments mostly as long as wide or slightly longer than wide. Terminal antennomere conspicuous. Antennae inserted at the lower line of compound eyes. Supraclypeal area shorter than clypeus and narrow. Face sculpture reticulate. Scrobe without a median longitudinal sulcus.

Mesosoma. Pronotum sculpture reticulate. Pronotum elongated, nearly twice as long as high in lateral view. Mesoscutum and mesoscutellum sculpture reticulate. Notauli complete. Frenal sulcus crenulated. Metascutellum long, rectangular to trapezoidal.

Figure 1. *Conidarnes achterbergi* sp. n., female. **A** *habitus* lateral view **B** antenna **C** antenna, detail.

Anterior margin of propodeum slightly crenulated. Propodeum sculpture reticulate. Propodeum without a median line.
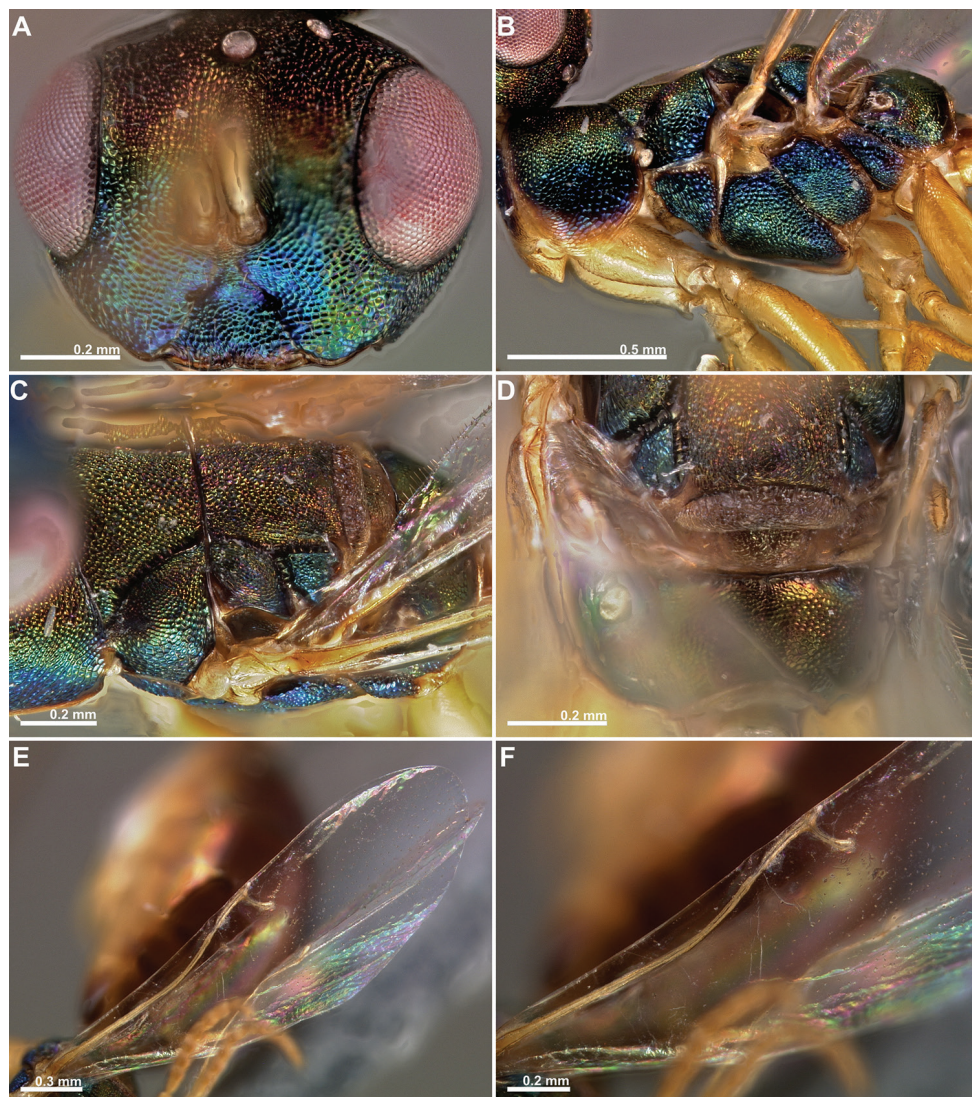
Metasoma. Length of the ovipositor sheaths 1.7× body length.

Male. Unknown.

Etymology. The species is dedicated to our colleague and renowned specialist of Hymenoptera, Kees van Achterberg who collected the holotype.

Biology. Unknown

Figure 2. *Conidarnes achterbergi* sp. n. female. **A** head in frontal view **B** mesosoma in lateral view **C** mesosoma in dorsal view **D** propodeum and terminal mesosoma in dorsal view **E** wing **F** detail of venation.

Conidarnes bergi Farache & Rasplus, sp. n.

http://zoobank.org/847475AA-A6A3-4DE6-8210-890517AAB4BB
Figs 3, 4, 5

Holotype. ♀: **INDONESIA: Java:** Gunung Tjibodas, –6.88° 106.65°, 530m, 19.XI.1954, van der Vecht J., ex *F. involucrata*, Wiebes Coll. n° 114 (RMNH).

Paratypes. 8♀, 8♂: **INDONESIA: Java:** Gunung Tjibodas, –6.88° 106.65°, 530m, 19.XI.1954, van der Vecht J., ex *F. involucrata*, Wiebes Coll. n° 114,
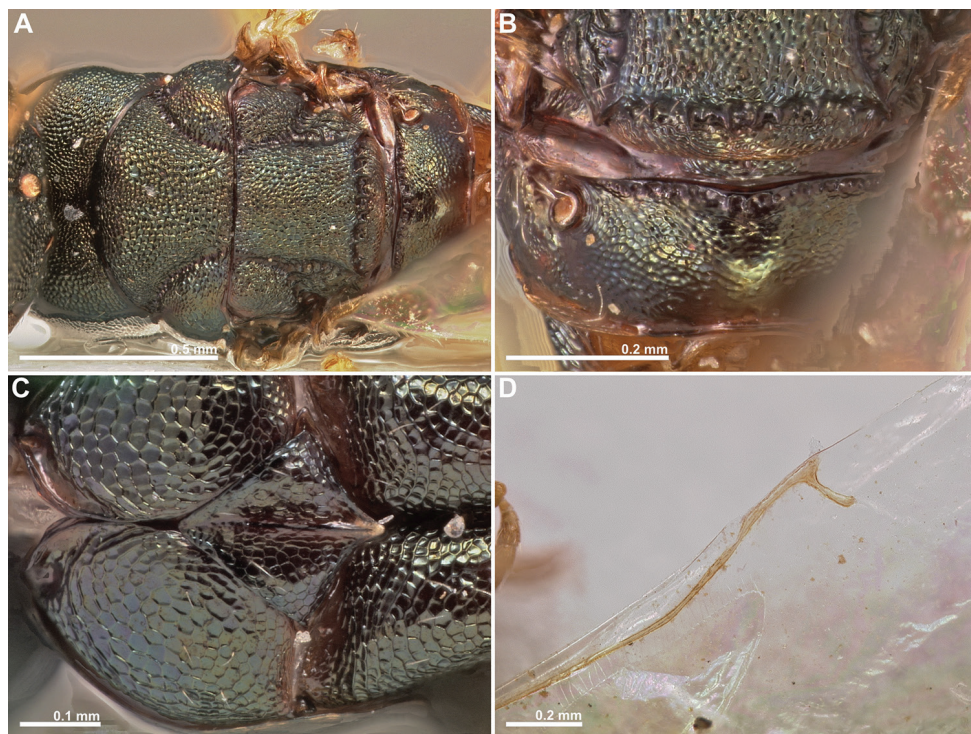
Figure 3. *Conidarnes bergi* sp. n. female. **A** *habitus* lateral view **B** antenna **C** antenna, detail **D** head in frontal view **E** head in dorsal view **F** mesosoma in lateral view.

19.XI.1954, de Gunst JH, ex *F. involucrata*, Wiebes Coll. n° 116 & 5103 (5♀, 5♂ RMNH; 2♀, 2♂ CBGP; 1♀, 1♂ RPSP).

Diagnosis. Antennae inserted at the lower line of compound eyes. Supraclypeal area shorter than clypeus. Supraclypeal area narrow. Mesoscutum and mesoscutellum sculpture reticulate. Prosternal posterior margin medially acute. Propodeum without a median line. Length of the ovipositor sheaths 0.46× body length.

Figure 4. *Conidarnes bergi* sp. n. female. **A** mesosoma in dorsal view **B** propodeum and terminal mesosoma in dorsal view **C** prosternum **D** detail of venation.

Description. *Female. Size and colour* Body length 2.8 mm. Length of the ovipositor sheaths 1.3 mm. Head and mesosoma black, slightly green. Metallic lustre faint. Antennae and legs yellow, coxae concolorous with mesosoma. Metasoma brown.

Head. Scape 5× as long as wide. Antenna with two anelli. Proximal anellus longer than distal anellus. Funicular segments mostly as long as wide or slightly longer than wide. Terminal antennomere inconspicuous. Antennae inserted at the lower line of compound eyes. Supraclypeal area shorter than clypeus, narrow. Face sculpture reticulate. Scrobe without a median longitudinal sulcus.

Mesosoma. Pronotum sculpture reticulate. Pronotum elongated, nearly twice as long as high in lateral view. Prosternal posterior margin medially acute. Mesoscutum and mesoscutellum sculpture reticulate. Notauli complete. Frenal sulcus crenulated. Metascutellum short, inconspicuous. Anterior margin of propodeum crenulated. Propodeum sculpture reticulate. Propodeum without a median line.

Metasoma. Length of the ovipositor sheaths 0.46× body length.

Male. Similar to female, except the following characters: Head and mesosoma darker. Legs browner. Pedicel and funicular segments more elongated. Antenna more setose. Pronotum more elongated.

Figure 5. *Conidarnes bergi* sp. n. male. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** mesosoma in dorsal view.

Etymology. The specific name is a tribute to our colleague and friend Kees Berg (2 July 1934–31 August 2012), for his excellent and unparalleled work on the taxonomy of fig trees.

Biology. Reared from syconia of *Ficus involucrata* Blume.

Conidarnes laevis Farache & Rasplus, sp. n.
http://zoobank.org/D8A7DDA5-5D19-4BC8-8A62-C5300ECCF9A6
Fig. 6

Holotype. ♀: **INDONESIA: E. Kalimantan:** Kutai Nature Reserve, 0.37° 117.27°, 5m, 1978, Leighton, ex *F. kerkhoveni*, Wiebes Coll. n° 3950 (RMNH).

Diagnosis. Head, mesosoma, and metasoma mostly brown. Upper face smooth; lower face reticulate. Mesoscutum and mesoscutellum sculpture mostly smooth. Notauli incomplete. Frenal sulcus smooth. Length of the ovipositor sheaths 1× body length.

Description. *Female. Size and colour.* Body length 1.7 mm. Length of the ovipositor sheaths 1.7 mm. Antennae yellow. Head dark brown, with metallic green lustre,
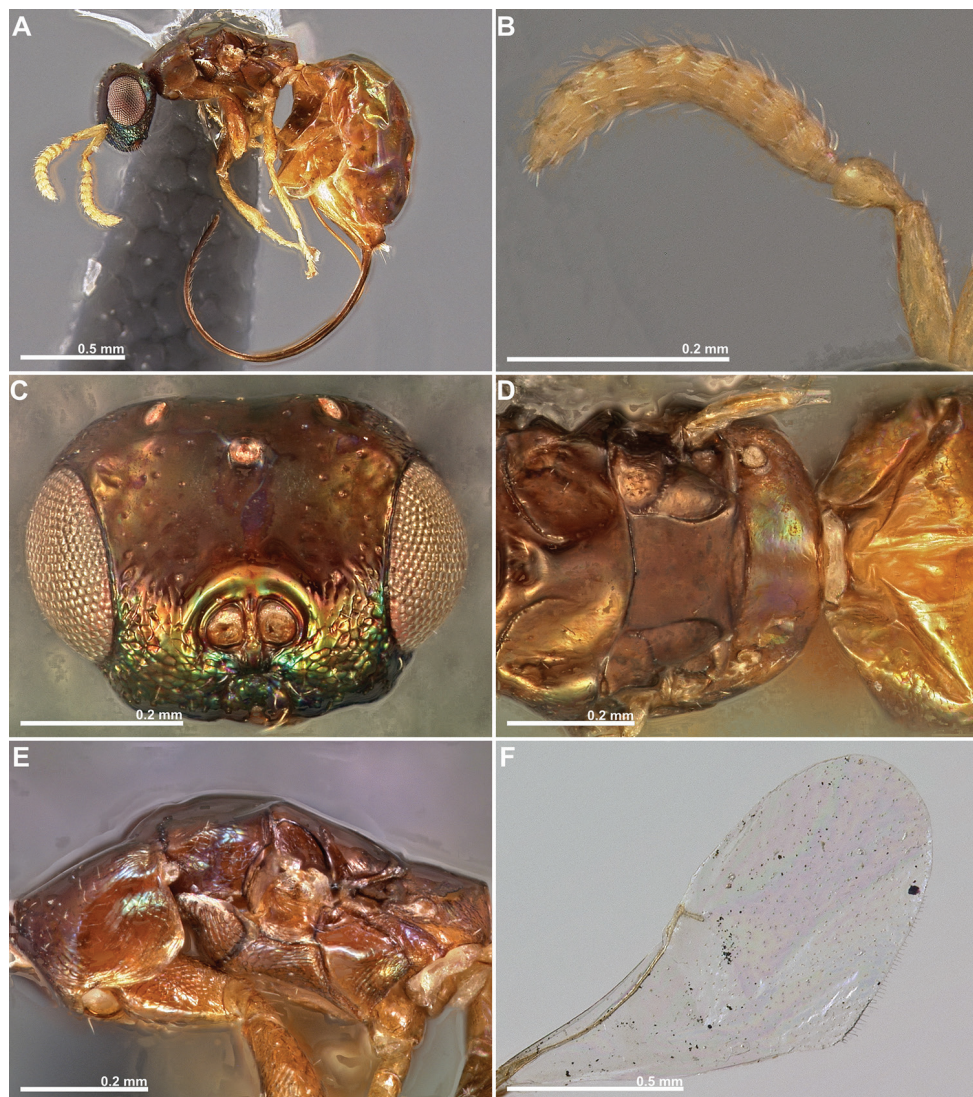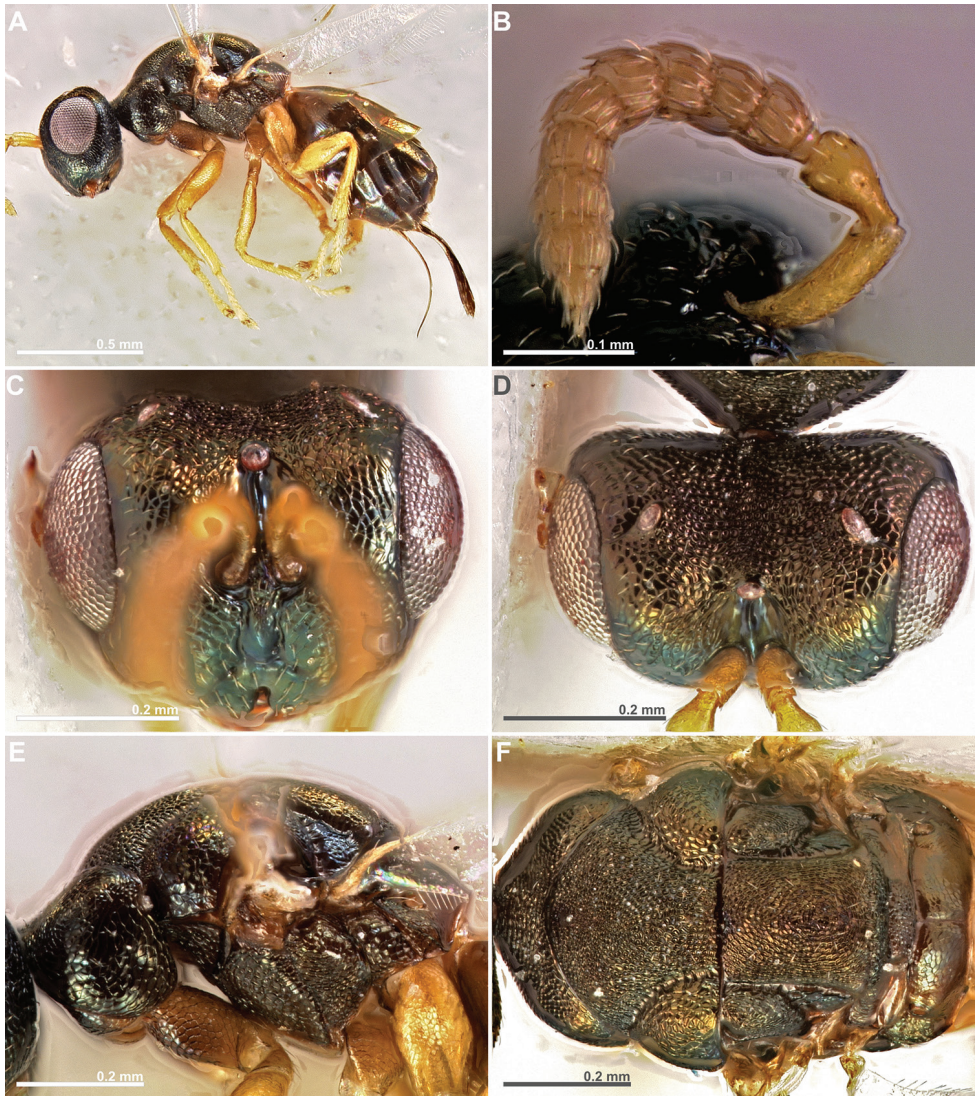
Figure 6. *Conidarnes laevis* sp. n. female. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** mesosoma in dorsal view (excluding pronotum) **E** mesosoma in lateral view **F** wing. Images **A, B, C, D**, and **F** by Gunther Fleck.

mostly at the lower face. Mesosoma and metasoma brown. Legs proximally brown, tibiae and tarsi yellow.

Head. Scape 3.5× as long as wide. Antenna with two anelli. Proximal anellus shorter than distal anellus. Funicular segments mostly transverse. Terminal antennomere inconspicuous. Antennae inserted at the lower line of compound eyes. Supraclypeal area shorter than clypeus, or inconspicuous, narrow. Upper face smooth; lower face reticulate. Scrobe without a median longitudinal sulcus.
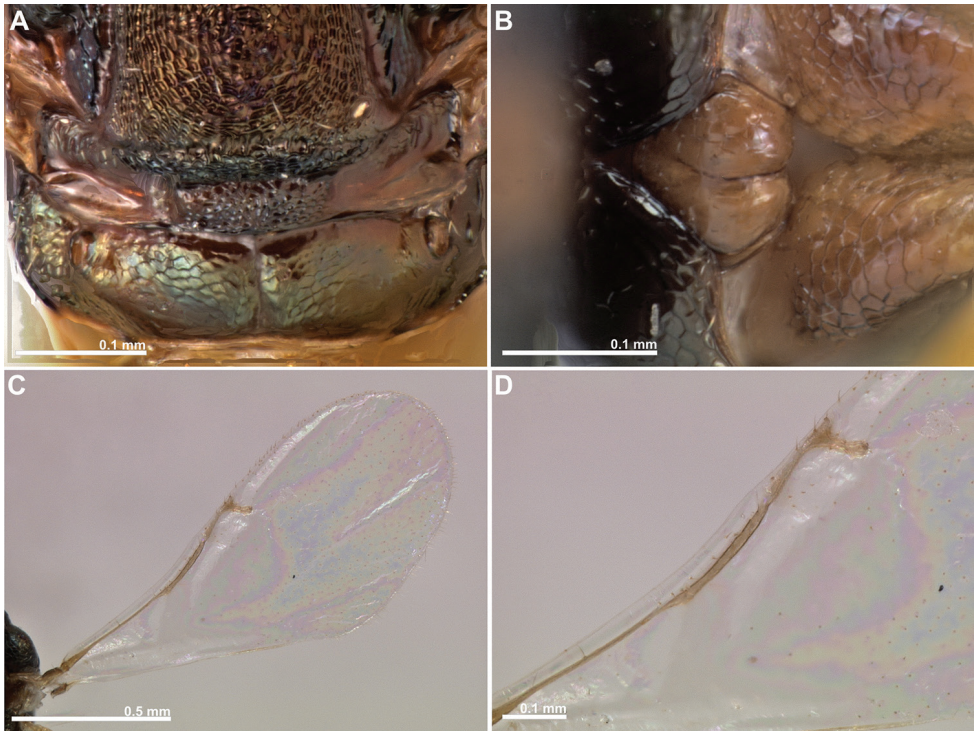
Figure 7. *Conidarnes santineloi* sp. n. female. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** head in dorsal view **E** mesosoma in lateral view **F** mesosoma in dorsal view.

Mesosoma. Pronotum sculpture mostly smooth, slightly engraved. Pronotum elongated, nearly twice as long as high in lateral view. Mesoscutum and mesoscutellum sculpture mostly smooth. Notauli incomplete. Frenal sulcus smooth. Metascutellum short, inconspicuous. Anterior margin of propodeum smooth. Propodeum sculpture smooth. Propodeum without a median line.

Metasoma. Length of the ovipositor sheaths 1× body length.

Male. Unknown.

Figure 8. *Conidarnes santineloi* sp. n. female. **A** propodeum and terminal mesosoma in dorsal view **B** prosternum **C** wing **D** detail of venation.

Etymology. The specific name refers to the smooth body sculpture observed in this species.

Biology. Reared from syconia of *Ficus kerkhovenii* Koord. & Valeton.

Note. This species presents unique characters, such as a smooth body with no sculpture and an elongated mesosoma. These characters are mostly associated to galler fig wasps that enters the syconium through the ostiole (Cruaud et al. 2011b). Consequently, we speculate that this species may be an ostiolar galler.
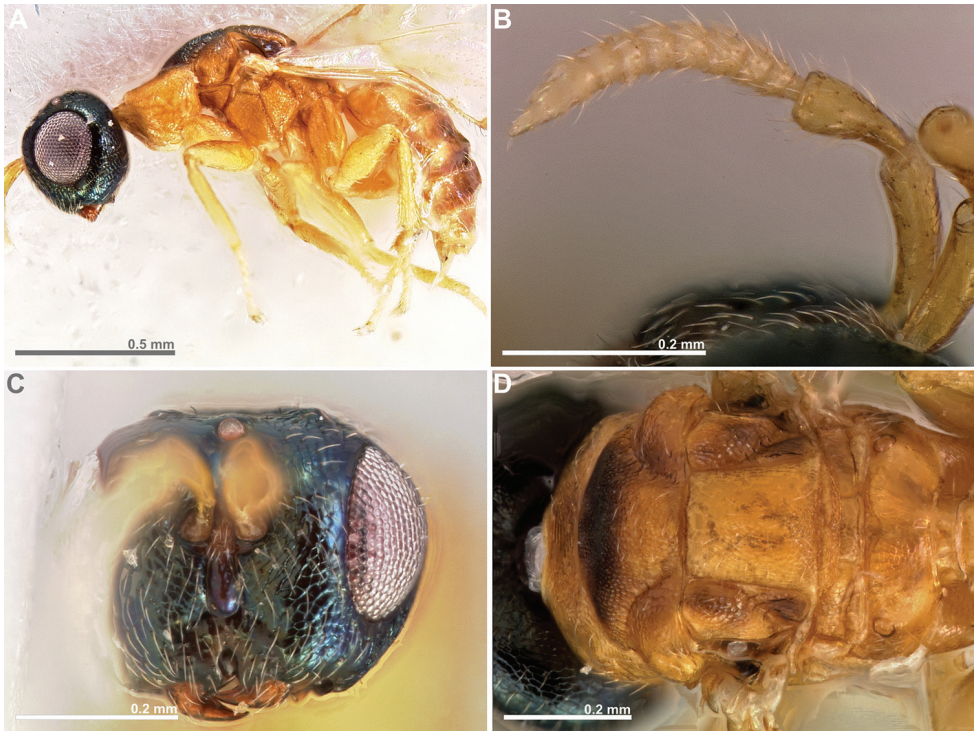
Conidarnes santineloi **Farache & Rasplus, sp. n.**
http://zoobank.org/DD49490F-74E2-4A9E-9B24-62145DCBAC52
Figs 7, 8, 9

Holotype. ♀: **BRUNEI:** Temburong National Park, 4.554° 115.157°, 100m, 25.XI.1996, Rasplus J.Y., ex *F. pallescens*, n° JRAS00114 (CBGP).

Paratypes. 9♀, 5♂: same locality and information as holotype (7♀, 3♂ CBGP; 1♀, 1♂ SAMC; 1♀, 1♂ RPSP).

Figure 9. *Conidarnes santineloi* sp. n. male. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** mesosoma in dorsal view.

Diagnosis. Antenna with one anellus. Funicular segments mostly transverse. Mesoscutum and mesoscutellum sculpture reticulate. Prosternal posterior margin not medially acute. Propodeum with a depressed median line. Length of the ovipositor sheaths 0.3× body length.

Description. *Female. Size and colour.* Body length 1.6 mm. Length of the ovipositor sheaths 0.45 mm. Head, mesosoma, and metasoma black, slightly green. Metallic lustre faint. Antennae and legs yellow, forecoxae brown.

Head. Scape 3.5× as long as wide. Antenna with one anellus. Funicular segments mostly transverse. Terminal antennomere inconspicuous. Antennae inserted just below the middle line of compound eyes. Supraclypeal area higher than clypeus, wide. Face sculpture reticulate. Scrobe without a median longitudinal sulcus.

Mesosoma. Pronotum sculpture reticulate. Pronotum not elongated, 1.5× as long as wide in lateral view or less. Prosternal posterior margin not medially acute. Mesoscutum and mesoscutellum sculpture reticulate. Notauli complete. Frenal sulcus crenulated. Metascutellum long, rectangular to trapezoidal. Anterior margin of propodeum smooth. Propodeum sculpture slightly reticulate to smooth. Propodeum with a depressed median line.

Metasoma. Length of the ovipositor sheaths 0.3× body length.

Male. Similar to female except the following characters: Mesosoma and metasoma yellow. Mesoscutum, axillae, axillulae, and dorsal metasoma partially brown black, with faint metallic lustre. Legs completely yellow. Pedicel and funicular segments slender. Antenna more setose. Pronotum slender.

Etymology. The specific name is dedicated to our friend and colleague Rodrigo Augusto Santinelo Pereira due to his excellent and pioneering work on fig wasps and *Ficus* in Brazil.

Biology. Collected from syconia of *Ficus pallescens* L., the form with small leaves (see Berg and Corner 2005).

Conidarnes subtectae Farache & Rasplus, sp. n.
urn:lsid:zoobank.org:act:1843245F-8200-48ED-A02C-0B7460D740A9
Figs 10, 11

Holotype. ♀: **BRUNEI:** Temburong, Kuala Belalong, 4.538° 115.159°, 105m, 23.IV.1997, Hossaert-Mckey M., ex *F. subtecta*, n° JRAS00117 (CBGP).

Diagnosis. Antenna with two anelli. Funicular segments mostly as long as wide or slightly longer than wide. Antennae inserted just below the middle line of compound eyes. Supraclypeal area wide. Mesoscutum and mesoscutellum reticulate. Length of the ovipositor sheaths 0.9× body length.

Description. *Female. Size and colour.* Body length 1.8 mm. Length of the ovipositor sheaths 1.6 mm. Antennae yellow. Head and mesosoma black, with faint blue, green, and orange metallic lustre. Legs mostly yellow distally. Coxae almost concolorous with body. Femora yellow brown. Metasoma dark brown.

Head. Scape 4.8× as long as wide. Antenna with two anelli. Proximal anellus longer than distal anellus. Funicular segments mostly as long as wide or slightly longer than wide. Terminal antennomere conspicuous. Antennae inserted just below the middle line of compound eyes. Supraclypeal area higher than clypeus, and wide. Face sculpture reticulate. Scrobe without a median longitudinal sulcus.

Mesosoma. Pronotum sculpture reticulate. Pronotum elongated, nearly twice as long as high in lateral view. Mesoscutum and mesoscutellum sculpture reticulate. Notauli complete. Frenal sulcus crenulated. Metascutellum long, rectangular to trapezoidal. Anterior margin of propodeum crenulated. Propodeum sculpture reticulate. Propodeum with a depressed median line.
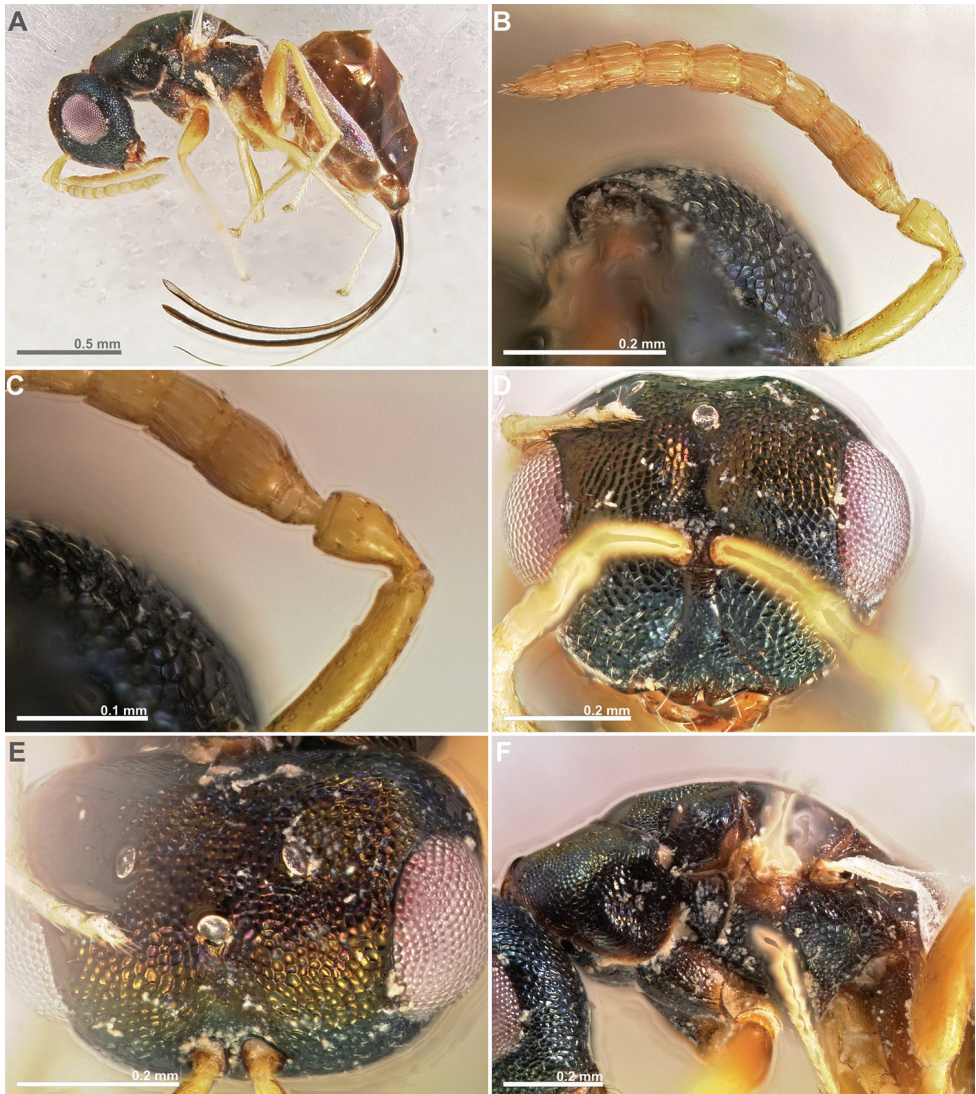
Metasoma. Length of the ovipositor sheaths 0.9× body length.

Male. Unknown.

Etymology. The specific name refers to the host fig species.

**Biology.** Reared from syconia of *Ficus subtecta* Corner

Figure 10. *Conidarnes subtectae* sp. n. female. **A** *habitus* lateral view **B** antenna **C** antenna, detail **D** head in frontal view **E** head in dorsal view **F** mesosoma in lateral view.

Conidarnes sulcata Farache & Rasplus, sp. n.
http://zoobank.org/4584C240-8A1A-4722-9BC7-8B18DDFC6833
Figs 12, 13

Holotype. ♀: **CHINA: Yunnan:** Luxi county, Mang Hai town, 24.53° 102.54°, 1750m, 28.IV.2006, Rasplus J.Y.; Peng Y.Q. & Yang D.R., ex *F. altissima*, n° JRAS01616_04 (CBGP).
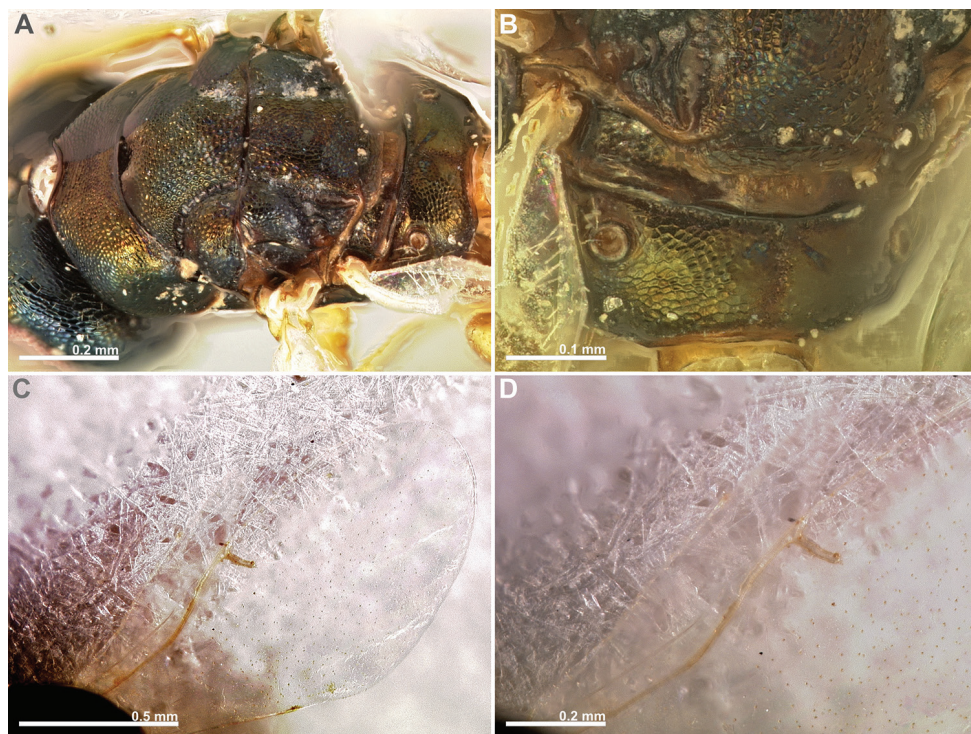
Figure 11. *Conidarnes subtectae* sp. n. female. **A** mesosoma in dorsal view **B** propodeum and terminal mesosoma in dorsal view **C** wing **D** detail of venation.

Paratypes. 7♂: **CHINA: Yunnan:** Cheng Zhi village, 21.92° 101.24°, 540m, 6♂, 9.IV.2002, Gu H.Y. & Rasplus J.Y., ex *F. altissima*, n° JRAS0875 (5 ♂ CBGP; 1♂ SAMC); **Yunnan:** Luxi county, Mang Hai town 24.53° 102.54°, 1750m, 1♂, 28.IV.2006, Rasplus J.Y.; Peng Y.Q. & Yang D.R., ex *F. altissima*, n° JRAS01616_04 (CBGP).

Diagnosis. Scrobe with a median longitudinal sulcus, extending from median ocellus to interantennal area. Mesoscutum and mesoscutellum sculpture mostly smooth. Lateral area of the mesoscutum mostly engraved reticulate. Propodeum sculpture smooth, slightly engraved alutaceous near spiracles. Propodeum without a median line. Length of the ovipositor sheaths 1× body length.

Description. *Female. Size and colour.* Body length 2.7 mm. Length of the ovipositor sheaths 2.6 mm. Antennae yellow. Head with metallic lustre, mostly green, slightly orange and blue. Mesosoma mostly brown, with faint metallic lustre, green and blue. Legs mostly brown, tarsal segments and foretibia yellow. Metasoma dark brown.

Head. Scape 4.6× as long as wide. Antenna with two anelli. Proximal anellus nearly as long as distal anellus. Funicular segments mostly transverse. Terminal antennomere conspicuous. Antennae inserted at the lower line of compound eyes. Supraclypeal area inconspicuous. Face sculpture mostly reticulate, smooth near scrobe. Scrobe with a median longitudinal sulcus, extending from median ocellus to interantennal area.
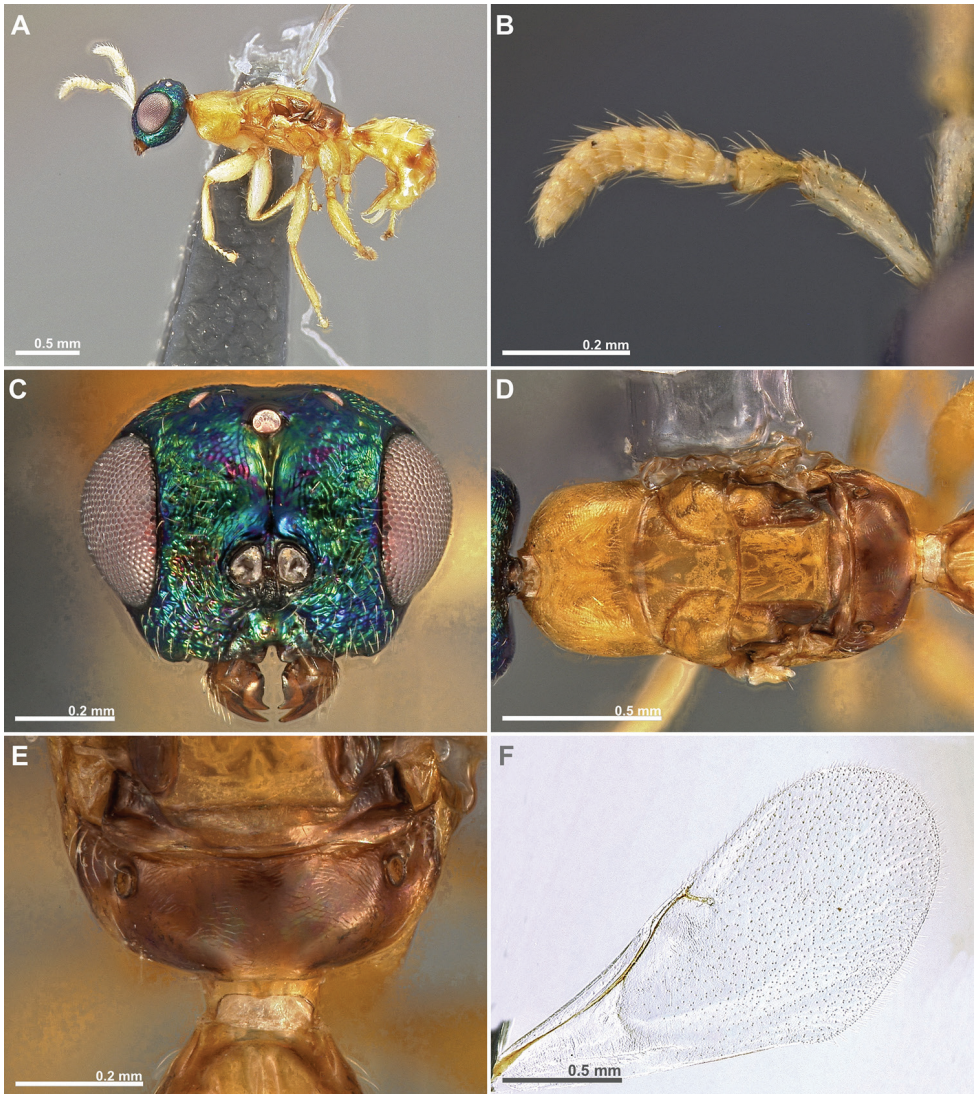
Figure 12. *Conidarnes sulcata* sp. n. female. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** mesosoma in dorsal view **E** propodeum and terminal mesosoma in dorsal view **F** detail of venation. Images by Gunther Fleck.

Mesosoma. Pronotum sculpture alutaceous, engraved. Pronotum elongated, nearly twice as long as high in lateral view. Prosternal posterior margin medially acute. Mesoscutum and mesoscutellum sculpture mostly smooth. Lateral area of the mesoscutum mostly engraved reticulate. Notauli complete. Frenal sulcus smooth. Metascutellum as long as frenum, smooth, and not well delimited laterally. Anterior margin of propodeum smooth. Propodeum sculpture smooth, slightly engraved alutaceous near spiracles. Propodeum without a median line.

Figure 13. *Conidarnes sulcata* sp. n. male. **A** *habitus* lateral view **B** antenna **C** head in frontal view **D** mesosoma in dorsal view **E** propodeum and terminal mesosoma in dorsal view **F** wing. Photographs by Gunther Fleck.

Metasoma. Length of the ovipositor sheaths 1× body length.

Male. Similar to female except the following characters: Mesosoma and metasoma mostly yellow. Axillulae, metanotum, and propodeum mostly brown. Some metasomal segments slightly brown dorsally. Scape and pedicel shorter, funicular segments more transverse. Antenna more setose.

Etymology. The specific name refers to the longitudinal sulcus separating the scrobal cavity in this species.

Biology. Reared from syconia of *Ficus altissima* Blume.

Comments. *Conidarnes sulcata* was included in the phylogenetic analyses by Cruaud et al. (2011b). It was referred to as *Conidarnes* ex *F. altissima* (China) 1616_04w01x. The following markers are available in GenBank for this species: COI (JN001522.1), CytB (JN001596.1), EF1a (JN001659.1), and rRNA 28S (JN001493.1).

Conidarnes sumatranae Farache & Rasplus, sp. n.
http://zoobank.org/105F5966-EDA7-483F-9908-50A47BBE8A04
Figs 14, 15

Holotype. ♀: **INDONESIA: Sulawesi:** Pattunuang, −5.059° 119.718°, 180m, 27.VIII.2007, Rasplus J.Y., ex *F. sumatrana*, n° JRAS02085_0202 (CBGP).

Paratypes. 1♂: same locality and information as holotype, n°JRAS02085_0201 (CBGP).

Diagnosis. Antennae inserted near the middle line of compound eyes. Funicular segments mostly as long as wide or slightly longer than wide. Mesoscutum and mesoscutellum sculpture reticulate. Propodeum with a reticulate median line, slightly striate, and thicker near anterior margin. Length of the ovipositor sheaths 0.4× body length.

Description. *Female. Size and colour.* Body length 1.9 mm. Length of the ovipositor sheaths 0.8 mm. Antennae yellow. Head and mesosoma black, with green and blue metallic lustre. Legs mostly yellow, forecoxae concolorous with body. Hindcoxae proximally concolorous with body. Metasoma dark brown.

Head. Scape 3.5× as long as wide. Antenna with two anelli. Proximal anellus longer than distal anellus. Funicular segments mostly as long as wide or slightly longer than wide. Terminal antennomere inconspicuous. Antennae inserted near the middle line of compound eyes. Supraclypeal area higher than clypeus and narrow. Face sculpture reticulate. Scrobe without a median longitudinal sulcus.

Mesosoma. Pronotum sculpture reticulate. Pronotum elongated, nearly twice as long as high in lateral view. Mesoscutum and mesoscutellum sculpture reticulate. Notauli complete. Frenal sulcus crenulated. Metascutellum long, rectangular to trapezoidal. Anterior margin of propodeum crenulated. Propodeum sculpture reticulate. Propodeum with a reticulate median line, slightly striate, and thicker near anterior margin.

Metasoma. Length of the ovipositor sheaths 0.4× body length.

Male. Similar to female, but slightly smaller.

Etymology. The specific name refers to the host species.

Biology. Reared from syconia of *Ficus sumatrana* Miq.

Comments. *Conidarnes sumatranae* was included in several phylogenetic analyses (Cruaud et al. 2011a; Cruaud et al. 2011b; Farache et al. 2013) and was referred as *Conidarnes* sp. ex *F. sumatrana* 2085_02w01a or as Undescribed genus sp. ex. *F. sumatrana* (2085_02w01a). The following molecular markers are available in GenBank for this species: COI (HM770620.1), CytB (HM770576.1), EF1a (HM770522.1), and rRNA 28S (HM770682.1), they were sequenced from the male paratype that has been subsequently dried and mounted on card.

Figure 14. *Conidarnes sumatranae* sp. n. female. **A** *habitus* lateral view **B** antenna **C** head in frontal view.

## *Conidarnes* sp. ex *Ficus sundaica*
Fig. 16

Material examined. 5♂: **INDONESIA: E. Kalimantan:** Kutai Nature Reserve, 0.37° 117.27°, 1978, Bingham M., ex *F. sundaica* Bl. v. *beccariana* (King) det. Corner, Wiebes Coll. N°3543 (RMNH).

Description. *Female.* Unknown.

Male. Size and colour. Body length 3.1 mm. Antennae yellow orange. Head and mesosoma mostly black, with metallic blue lustre. Legs mostly dark brown, proximally darker. Metasoma brown.
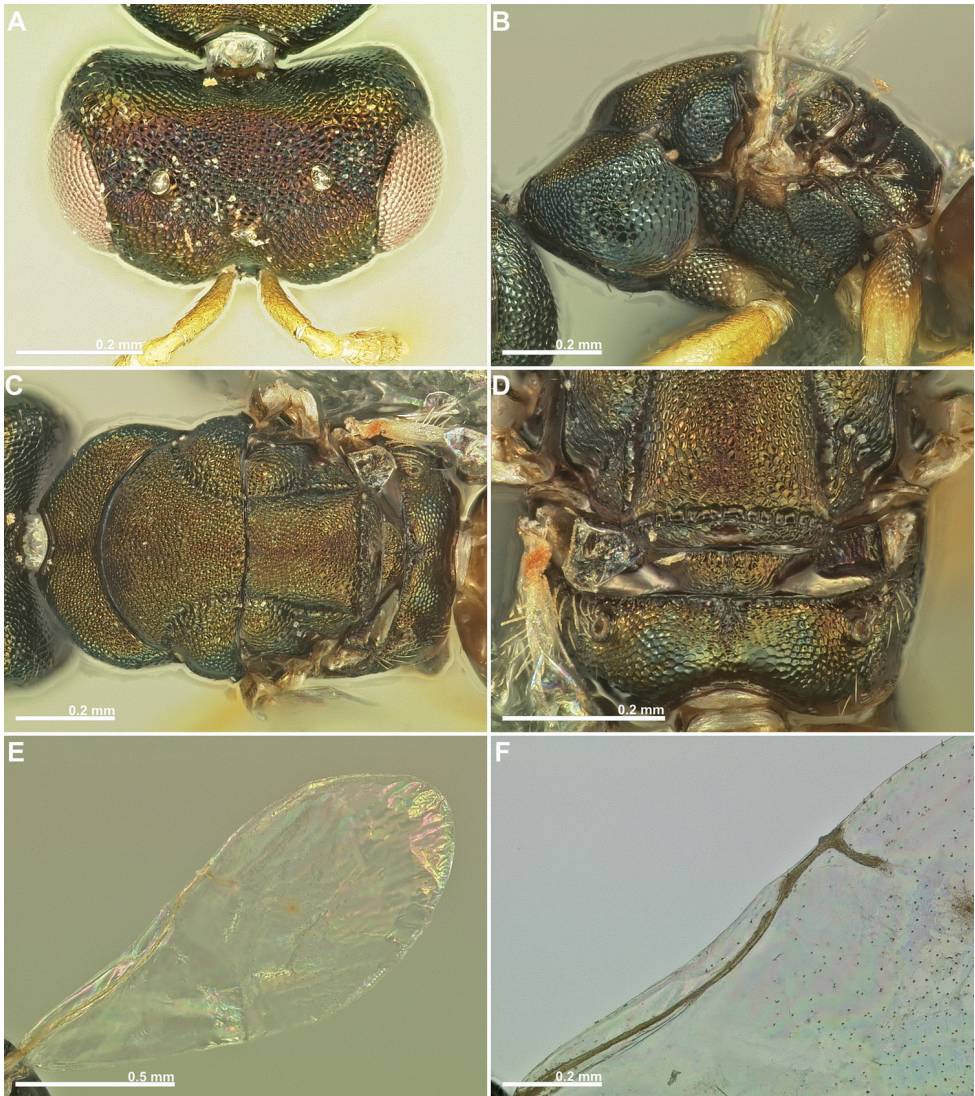
Figure 15. *Conidarnes sumatranae* sp. n. female. **A** head in dorsal view **B** mesosoma in lateral view **C** mesosoma in dorsal view **D** propodeum and terminal mesosoma in dorsal view **E** wing **F** detail of venation.

Head. Scape 5.3× as long as wide. Antennae inserted just below the middle line of compound eyes. Supraclypeal area shorter than clypeus and narrow. Face sculpture reticulate. Scrobe with a median longitudinal sulcus, extending from median ocellus to interantennal area.

Mesosoma. Pronotum sculpture alutaceous, engraved. Pronotum elongated, nearly twice as long as high in lateral view. Mesoscutum and mesoscutellum sculpture reticu-

Figure 16. *Conidarnes* sp. ex *Ficus sundaica* male. **A** *habitus* lateral view **B** head in frontal view **C** mesosoma in lateral view **D** mesosoma in dorsal view **E** wing **F** detail of venation.

late. Notauli complete. Frenal sulcus crenulated. Metascutellum long, rectangular to trapezoidal. Anterior margin of propodeum crenulated. Propodeum sculpture slightly reticulate to smooth. Propodeum without a median line.

Biology. This species was reared from *Ficus sundaica* Blume v. *beccariana* (King).

Comments. We have examined only males, but they clearly belong to an undescribed species. Since we described *Conidarnes* species mostly based on females, we prefer not to describe this species until more specimens are found.

Discussion

In this study, we describe a new oriental genus of Sycophaginae that includes seven new species. *Conidarnes* can easily be assigned to Sycophaginae due to the presence of a square mesoscutellum and the morphology of the terminal gastral tergites/epipygium, which are synapomorphies of the subfamily (Cruaud et al. 2011b; Rasplus and Soldati 2005). The assignment of *Conidarnes* to Sycophaginae is also corroborated by previous phylogenetic analyses (Cruaud et al. 2011a; Cruaud et al. 2011b). Among the Sycophaginae, *Conidarnes* is uniquely defined by the following combination of characters: toruli contiguous; antennae inserted at, or below, the median line of compound eyes; malar sulcus absent; petiole very short, transverse.

Phylogenetically, *Conidarnes* is nested within a clade including *Pseudidarnes* and *Anidarnes*. Species belonging to this clade are large gall inducers (Cruaud et al. 2011b). This biology seems to be shared by all members of the clade, a life-history strategy that is also found in the *Idarnes incertus* species group and in a few *Sycophaga* species (Cruaud et al. 2011b). Large gall inducers oviposit early during fig development, species are overall larger and have shorter ovipositors than the other Sycophaginae species developing in the same fig (Cruaud et al. 2011b). Based on morphology and phylogenetic relationships, most *Conidarnes* species seem to be large gall-inducers, which oviposit through the syconium wall several days before pollination, though this still needs to be confirmed by behavioural observations. One peculiar species, *Conidarnes laevis*, exhibits a rather flattened and smooth body (Figs 6A, E). Such morphology may indicate that females enter the fig through the ostiole. If this biology is confirmed by field observations, it would be a second independent case of an ostiolar Sycophaginae besides species of the *Sycophaga sycomori* species group that are associated with *Ficus* subgenus *Sycomorus* in the Afrotropical region (Galil et al. 1970).

Conidarnes species are restricted to the Oriental region. Only one species was sampled in continental Asia (*C. sulcata*, from Xishuangbanna in southwest China), whereas all other species were sampled in the insular region of Southeast Asia: five species in Borneo (*C. achterbergi*, *C. laevis*, *C. santineloi*, *C. subtectae*, and an undescribed species ex *Ficus sundaica*), one in Java (*C. bergi*) and one in Sulawesi (*C. sumatranae*).

The distribution of *Conidarnes* does not overlap with distribution of the two other genera belonging to the same clade. Indeed, *Anidarnes* is restricted to America (Farache et al. 2013), and *Pseudidarnes* occurs in Papua New Guinea, Australia, and the Solomon Islands (Farache and Rasplus 2014). This pattern corresponds to the distribution of their host *Ficus* species: section *Americana* (host of *Anidarnes*) is Neotropical, whereas section *Malvanthera* (host of *Pseudidarnes*) mostly occurs in Australia and New Guinea. *Conidarnes* is strictly associated with *Conosycea*, a section of figs occurring from India to Solomon Islands, with two species reaching Madagascar (Berg 1989; Farache et al. 2013; Farache and Rasplus 2014). The section *Conosycea* probably originated in continental Eurasia and subsequently spread through the islands of Southeast Asia, reaching Australasia and Madagascar (Cruaud et al. 2012).

Another characteristic of Sycophaginae species belonging to the clade of large gall inducers is that they are rare and globally difficult to sample (Cruaud et al. 2011b). *Conidarnes* and *Pseudidarnes* species are among the rarest Sycophaginae (Cruaud et al. 2011b; Farache et al. 2013; Farache and Rasplus 2014). These characteristics plus the difficulty to find and sample ripening hemi-epiphytic stranglers (*Conosycea*) in the jungle explain why several species described here are only known from one or a few specimens. Sampling of *Conidarnes* is always extremely difficult and sporadic. To exemplify this point, we only sampled 6 males of *C. sulcata* despite collecting and opening about 5000 figs of *F. altissima* in southern China. Consequently, we only obtained sequences from a few of these species (three) and we therefore encourage extensive sampling of *Conosycea* figs to improve our knowledge of the genus.

Acknowledgements

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Supplementary material 1

Description characters and HAO codes for *Conidarnes*
Authors: Fernando Henrique Antoniolli Farache, Jean-Yves Rasplus
Data type: List of morphological characters
Explanation note: List of characters and states, with HAO portal codes. http://portal. hymao.org/projects/32/public/ontology

Supplementary material 2

LUCID Key for *Conidarnes*
Authors: Fernando Henrique Antoniolli Farache, Jean-Yves Rasplus
Data type: Key to species
Explanation note: Digital multi-entry key to species of *Conidarnes* in LUCID format: http://www.lucidcentral.com

RESEARCH ARTICLE

Description of *Pella tianmuensis* sp. n. from eastern China (Coleoptera, Staphylinidae, Aleocharinae)

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Abstract

Pella tianmuensis **sp. n.**, a myrmecophile associated with *Lasius* (*Dendrolasius*) *spathepus* Wheeler, 1910 in West Tianmushan Natural Reserve, Zhejiang, is described, illustrated and distinguished from its congeners.

Keywords

Introduction

The genus *Pella* Stephens, 1833 was previously represented by 63 species (Hlaváč et al. 2011, Song and Li 2013, Zheng and Zhao 2014), nine of which have been reported from China. In 2014, our team surveyed the staphylinid fauna of the West Tianmushan (Zhejiang, East China), and collected a series of an unidentified *Pella* species from a colony of the species of *Lasius* (*Dendrolasius*) *spathepus* Wheeler, 1910. An examination of this material revealed that the *Pella* species was undescribed.

Material and methods

Specimens were killed with ethyl acetate and preserved in 75% ethanol before dissection. Photos of the *habitus* were taken with a Canon EOS 70D with an MP-E 65 mm macro photo lens. Head length was measured from the clypeal anterior marginto the occipital constriction; elytral length at the suture from the apex of the scutellum to the elytral posterior margin.

The following abbreviations are used in the text: **BL**—body length, from the anterior margin of the labrum to the abdominal apex of tergite VIII; **FBL**—forebody length, from the clypeal anterior margin to the posterior margin of elytra; **PL**—length of the pronotum along midline; **HW**—width of the head across the eyes; **PW**—maximum width of the pronotum; **EL**—length of elytra from the apex of the scutellum to the posterior margin of the elytra; **EW**—maximum width of the elytra; **SL**—length of elytral suture.

All the types are deposited in the Insect Collection of Shanghai Normal University, Shanghai, China (SNUC).

Taxonomy

Pella tianmuensis sp. n.
http://zoobank.org/DFC82D87-3AEF-47FB-9B4A-2EEAA6CD12E6
Fig. 1

Diagnosis. The new species is characterized by dark coloration of body, bicoloured elytra (yellowish maculation extending from humeral angles to mesal area), absence of a sexual dimorphism of the head, a basally curved and apically obtuse (lateral view) ventral process of the aedeagus, and a pronounced and long crista apicalis of the aedeagus.

Type material (17 ♂♂, 27 ♀♀). Holotype: 1 ♂, labelled 'China: Zhejiang Prov., Lin'an City, W. Tianmushan (西天目山), nr. Kaishanlaodian (开山老殿), 30°20'45"N; 119°25'34"W, alt. 1200 m, 30.v.2014, Xiao-Bin Song & Liang Tang leg. // HOLOTYPE [red], *Pella tianmuensis* sp. n., Yan & Li det. 2015, SNUC'. Paratypes: 16 ♂♂, 27 ♀♀, same label data as holotype, all bearing the following label: 'PARATYPE [yellow], *Pella tianmuensis* sp. n., Yan & Li det. 2015, SNUC'.

Description. Body (Fig. 1A) length: 4.56–6.60 mm. Coloration: fore body black; elytra bicoloured, with yellowish maculation extending from humeral angles to mesal area; abdomen brownish-black, with posterior margins of segments yellowish-brown; legs and antennae dull-red.

Head (Fig. 1A) widest anteriorly; surface finely reticulate, covered with short golden setae; antennomeres VI–X distinctly transverse (Fig. 1B). Pronotum (Fig. 1A) 1.28 times as wide as long and 1.40 times as wide as head; widest approximately in anterior third, narrowed posteriorly; surface covered with short golden setae; hypomera fully visible in lateral view. Elytra (Fig. 1A) approximately 1.08 times as long as pronotum;
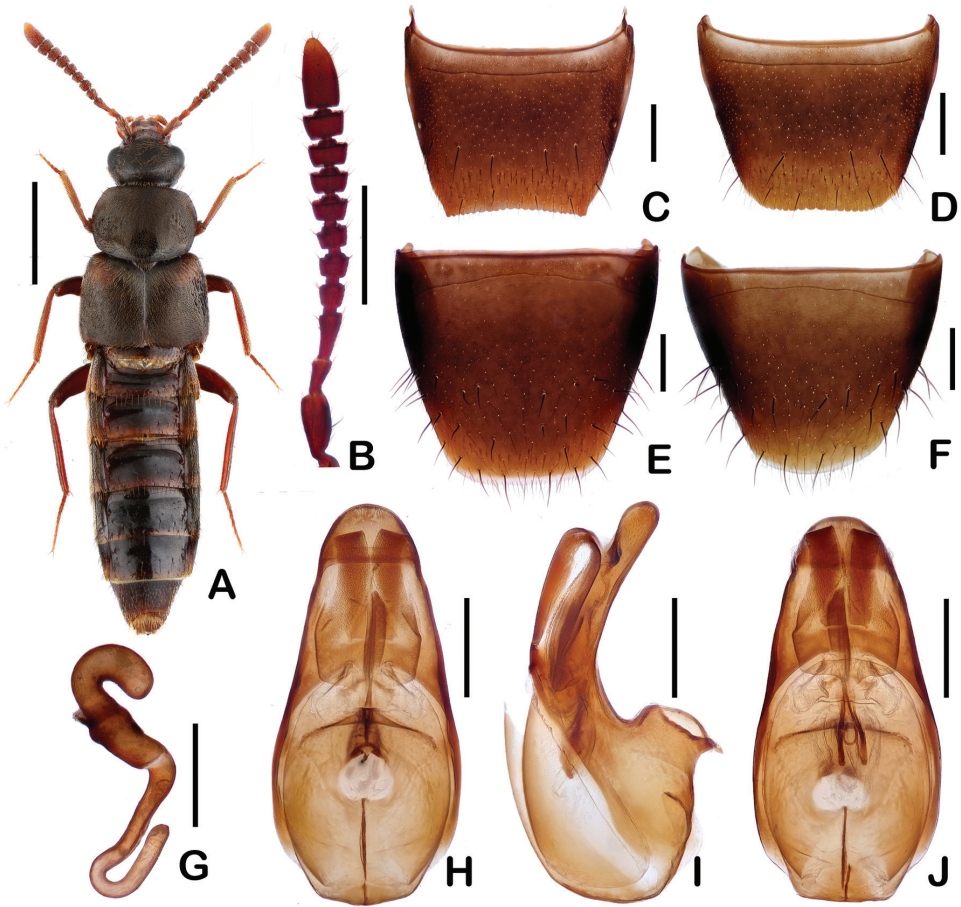
Figure 1. *Pella tianmuensis* sp. n. **A** Dorsal *habitus* **B** Antenna **C** Male tergite VIII **D** Female tergite VIII **E** Male sternite VIII **F** Female sternite VIII **G** Spermatheca **H** Aedeagus, in ventral view **I** Aedeagus, in lateral view **J** Aedeagus, in dorsal view. Scale bars: 2.0 mm (**A**); 0.5 mm (**B**); 0.2 mm (**C–J**).

covered with short golden setae; humeral angle with one macroseta. Hind wings fully developed. Abdomen (Fig. 1A) widest at segments III–IV; surface with transverse microsculpture.

Male. Tergite VIII (Fig. 1C) with posterior margin slightly emarginate, its emarginated apex weakly serrate; sternite VIII (Fig. 1E) with posterior margin rounded; median lobe of the aedeagus (Figs 1H–J) cone-shaped in ventral view; ventral process of aedeagus curved at base, obtuse at apex in lateral view; copulatory piece as in Fig. 1J.

Female: Tergite VIII (Fig. 1D) with posterior margin truncate and weakly crenate; sternite VIII (Fig. 1F) with 12 or 13 pairs of macrosetae. Spermatheca (Fig. 1G) coiled three times.

Measurements. BL: 4.56–6.56; FBL: 2.19–2.67; HW: 0.87–0.93; PL: 0.92–1.01; PW: 1.22–1.32; EL: 1.02–1.18; EW: 1.45–1.54; SL: 0.79–0.82.

Biological notes. Most material of the new species was taken by sifting mixed leaf litter around the nest of *Lasius* (*Dendrolasius*) *spathepus*, together with three species of *Homoeusa* Kraatz, 1858 and with *Dendrolasiophilus monstrotibialis* (Hlaváč, Sugaya & Zhou, 2002). At least three *Pella* and some *Homoeusa* beetles were observed walking along the ant trails. Approximately five *Pella* individuals were observed eating dead caterpillars together with *Lasius* workers.

Remarks. Based on the size of eyes, the shapes of the pronotum, the bicolored elytra, and the morphology of the aedeagal median lobe, *Pella tianmuensis* belongs to the *P. cognata* group, of which four species are known from China: *P. kishimotoi* Maruyama, 2006, *P. sichuanensis* Zheng & Zhao, 2014, *P. puetzi* Assing, 2009, and *P. maoershanensis* Song & Li, 2013. The new species is distinguished from *P. kishimotoi* by the broader and shorter ventral process of the aedeagus in ventral view; from *P. sichuanensis* by the darker color of the body, and by the length of the elytra slightly exceeding that of the pronotum (*P. sichuanensis*: EL/PL= 0.86); from *P. puetzi* and *P. maoershanensis* by the absence of a sexual dimorphism of the head and the different shape of the ventral process of the aedeagus, especially in lateral view.

Etymology. The specific epithet is derived from the type locality.

Acknowledgments

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